

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	472	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2003/02/10 08:32
2	L2	4827	trehalose	USPAT; US-PGPUB	2003/02/10 08:33
3	L3	49	1 and 2	USPAT; US-PGPUB	2003/02/10 08:33
4	L4	128	non adj reducing adj saccharide\$1	USPAT; US-PGPUB	2003/02/10 08:39
5	L5	21	1 and 4	USPAT; US-PGPUB	2003/02/10 08:40
6	L6	4851	\$trehalose	USPAT; US-PGPUB	2003/02/10 08:41
7	L7	49	1 and 6	USPAT; US-PGPUB	2003/02/10 08:41

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	472	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2003/02/10 08:32
2	L2	4827	trehalose	USPAT; US-PGPUB	2003/02/10 08:33
3	L3	49	1 and 2	USPAT; US-PGPUB	2003/02/10 08:33

PGPUB-DOCUMENT-NUMBER: 20030017573

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017573 A1

TITLE: Polymerase kappa compositions and methods thereof

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Friedberg, Errol C.	Dallas	TX	US	
Gerlach, Valerie	Branford	CT	US	
Feaver, William J.	Branford	CT	US	

APPL-NO: 09/ 971101

DATE FILED: October 4, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60238289 20001004 US

US-CL-CURRENT: 435/226,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

The present invention concerns compositions and methods involving mammalian polymerase kappa, an enzyme with limited fidelity and moderate processivity. Methods of modulating polymerase kappa activity, such as inhibiting or reducing its activity, as a means of effecting a cancer treatment or preventative agent are provided, both by itself and in combination with other anti-cancer therapies. Also described are methods of screening involving assaying for polymerase kappa activity or expression, in addition to methods of screening for modulators of polymerase kappa to identify anti-cancer compounds.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/238,289, filed Oct. 4, 2000, the entire disclosure of which is specifically incorporated herein by reference. The government may own rights in the present invention pursuant to grant numbers CA 75733 and CA69029 from the National Cancer Institute.

----- KWIC -----

Detail Description Paragraph - DETX:

[0054] The early stages of the evolutionary history of the UmuC/DinB

superfamily of TLS-associated polymerases are uncertain because of horizontal gene transfer and lineage-specific gene loss. The importance of these modes of evolution is supported both by the patchy distribution of these polymerases in bacteria and archaea (with only one archaeal member identified so far in the crenarchaeon Sulfolobus solfataricus (Kuleava et al., 1996)), and by the location of the umuC genes on plasmids and the uvrX gene in a bacteriophage. It appears that at least one gene coding for this type of polymerase was present at the base of the eukaryotic crown group, with an early duplication resulting in the emergence of the Rev1 and Rad30 (Pol.eta./iota.) families. A subsequent duplication, probably at an early stage of metazoan evolution, resulted in the divergence of pol .eta. and pol .iota..

#### Detail Description Paragraph - DETX:

[0109] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, gamma-interferon, GMCSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion also is contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20030003452

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030003452 A1

TITLE: High fidelity reverse transcriptases and uses thereof

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

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Potter, Robert Jason	Frederick	MD	US	
Rosenthal, Kim	Laytonsville	MD	US	

APPL-NO: 09/ 808124

DATE FILED: March 15, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60189454 20000315 US

US-CL-CURRENT: 435/6,435/199 ,435/320.1 ,435/325 ,435/69.1 ,435/91.2 ,536/23.2

ABSTRACT:

The invention relates to reverse transcriptases which have increased fidelity (or reduced misincorporation rate) and/or terminal deoxynucleotidyl transferase activity. In particular, the invention relates to a method of making such reverse transcriptases by modifying or mutating specified positions in the reverse transcriptases. The invention also relates to nucleic acid molecules containing the genes encoding the reverse transcriptases of the invention, to host cells containing such nucleic acid molecules and to methods to make the reverse transcriptases using the host cells. The reverse transcriptases of the invention are particularly suited for nucleic acid synthesis, sequencing, amplification and cDNA synthesis.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 60/189,454, filed Mar. 15, 2000, the contents of which are incorporated by reference herein in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0111] A variety of DNA polymerases are useful in accordance with the present invention. Such polymerases include, but are not limited to, *Thermus thermophilus* (Tth) DNA polymerase, *Thermus aquaticus* (Taq) DNA polymerase, *Thermotoga neapolitana* (Tne) DNA polymerase, *Thermotoga maritima* (Tma) DNA polymerase, *Thermococcus litoralis* (Tli or VENT.TM.) DNA polymerase, *Pyrococcus furiosus* (Pfu) DNA polymerase, DEEPVENT.TM. DNA polymerase, *Pyrococcus woosii* (Pwo) DNA polymerase, *Bacillus sterothermophilus* (Bst) DNA polymerase, *Bacillus caldophilus* (Bca) DNA polymerase, *Sulfolobus acidocaldarius* (Sac) DNA polymerase, *Thermoplasma acidophilum* (Tac) DNA polymerase, *Thermus flavus* (Tfi/Tub) DNA polymerase, *Thermus ruber* (Tru) DNA polymerase, *Thermus brockianus* (DYNAZYME.TM.) DNA polymerase, *Methanobacterium thermoautotrophicum* (Mth) DNA polymerase, *Mycobacterium* spp. DNA polymerase (Mtb, Mlep), and mutants, variants and derivatives thereof.

Detail Description Paragraph - DETX:

[0116] In addition to the enzyme components, the present compositions preferably comprise one or more buffers and cofactors necessary for synthesis of a nucleic acid molecule such as a cDNA molecule. Particularly preferred buffers for use in forming the present compositions are the acetate, sulfate, hydrochloride, phosphate or free acid forms of Tris-(hydroxymethyl)aminomethane (TRIS.RTM.), although alternative buffers of the same approximate ionic strength and pKa as TRIS.RTM. may be used with equivalent results. In addition to the buffer salts, cofactor salts such as those of potassium (preferably potassium chloride or potassium acetate) and magnesium (preferably magnesium chloride or magnesium acetate) are included in the compositions. Addition of one or more carbohydrates and/or sugars to the compositions and/or synthesis reaction mixtures may also be advantageous, to support enhanced stability of the compositions and/or reaction mixtures upon storage. Preferred such carbohydrates or sugars for inclusion in the compositions and/or synthesis reaction mixtures of the invention include, but are not limited to, sucrose, trehalose, and the like. Furthermore, such carbohydrates and/or sugars may be added to the storage buffers for the enzymes used in the production of the enzyme compositions and kits of the invention. Such carbohydrates and/or sugars are commercially available from a number of sources, including Sigma (St. Louis, Mo.).

Detail Description Paragraph - DETX:

[0122] In another aspect, the compositions and reverse transcriptases of the invention may be prepared and stored in dry form in the presence of one or more carbohydrates, sugars, or synthetic polymers. Preferred carbohydrates, sugars or polymers for the preparation of dried compositions or reverse transcriptases include, but are not limited to, sucrose, trehalose, and polyvinylpyrrolidone (PVP) or combinations thereof. See, e.g., U.S. Pat. Nos. 5,098,893, 4,891,319, and 5,556,771, the disclosures of which are entirely incorporated herein by reference. Such dried compositions and enzymes may be stored at various temperatures for extended times without significant deterioration of enzymes or components of the compositions of the invention. Preferably, the dried reverse transcriptases or compositions are stored at 4.degree. C. or at -20.degree. C.

PGPUB-DOCUMENT-NUMBER: 20020197698

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197698 A1

TITLE: Novel amylases

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Igarashi, Kazuaki	Haga-gun		JP	
Endo, Keiji	Haga-gun		JP	
Ozaki, Katsuya	Haga-gun		JP	

APPL-NO: 10/ 136272

DATE FILED: May 2, 2002

RELATED-US-APPL-DATA:

child 10136272 A1 20020502 parent division-of 09465519 19991216 US GRANTED  
parent-patent 6403355 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	10-362487	1998JP-10-362487	December 21, 1998
JP	10-362488	1998JP-10-362488	December 21, 1998

US-CL-CURRENT: 435/201,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

Liquefying alkaline amylases, each having residual activity not less than 70% when treated at pH 10 and 45.degree. C. for 30 minutes in the presence of 1 to 100 mM of EDTA or EGTA, are described. Also described are detergents comprising these amylases. In comparison with conventional amylases for detergents, the liquefying alkaline amylases of this invention have a high chelating-agent resisting performance.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0006] Among the liquefying amylases known to date, a liquefying .alpha.-amylase (WO90/11352) derived from the strain belonging to *Pyrococcus* sp. and an .alpha.-amylase (WO96/02633) which is derived from the strain belonging to *Sulfolobus* sp. and is effective in the liquefying step of a starch are free from the influence from a chelating agent. These enzymes however have the optimum acting pH in a range of 4 to 6 and 2.5 to 4.5, respectively and do not act in the alkaline range so that they are not suited as a component of a detergent.

#### Detail Description Table CWU - DETL:

1 TABLE 1 Strain KSM-K36 Stain KSM-K38 (a) Results of microscopic The strains K36 and K38 are bacilli having a size of 1.0 to 1.2 .mu.m .times. observation 2.4 to 5.4 .mu.m and 1.0 to 1.2 .mu.m .times. 1.8 to 3.8 .mu.m, respectively. They form an oval endospore (1.0 to 1.2 .mu.m .times. 1.2 to 1.4 micron) at the center or near the end of the cell. Positive in the Gram's stain. Having no acid resistance. (b) Growth in various media Since the present strain is alkaliphilic, 0.5% sodium carbonate is added to the medium employed in the following tests. Nutrient agar plate culture Good growth is observed. The Good growth is observed. The colony has a circular shape. It colony has a circular shape. It has a flat surface, but a rough has a flat surface and a smooth periphery. The color of the periphery. The color of the colony is pale earthlike color. colony is yellowish brown. Nutrient agar slant culture Growth is observed. Growth is observed. Nutrient broth culture Growth is observed. Growth is observed. Nutrient-gelatin stab culture Good growth is observed. No Good growth is observed. No liquefaction of gelatin is liquefaction of gelatin is observed. observed. Litmus milk No change is observed. No change is observed. (c) Physiological properties Reduction of a nitrate and Reduction of a nitrate is positive. Reduction of a nitrate is positive. denitrification reaction Denitrification reaction is Denitrification reaction is negative, negative. MR test Owing to the alkaline medium, Owing to the alkaline medium, judgment is impossible. judgment is impossible. V-P test Negative. Negative. Formation of indole Negative. Negative. Formation of hydrogen nitride Negative. Negative. Hydrolysis of starch Positive. Positive. Assimilation of citric acid It grows on a Christensen's It grows on a Christensen's medium, but not on a Koser's medium, but not on a Koser's medium and Simmon's medium. medium and Simmon's medium. Assimilation of an inorganic It assimilates a nitrate but not an It assimilates a nitrate but not an nitrogen source ammonium salt. ammonium salt. Formation of a colorant Formation of a pale yellow Negative. colorant on King's B medium. Urease Negative. Negative. Oxidase Negative. Negative. Catalase Positive. Positive. Range for growth Temperature range for growth is Temperature range for growth is 15 to 40.degree. C. The optimum growth 15 to 40.degree. C. The optimum growth temperature ranges from 30 to temperature is 30.degree. C. 37.degree. C. The pH range for growth is 9.0 to The pH range for growth is 8.0 to 11.0. The optimum growth pH is 11.0. The optimum growth pH is similar to the above. pH 10.0 to 11.0. Behavior to oxygen Aerophilic. Aerophilic. O-F test No growth is observed. No growth is observed. Assimilation of saccharides Assimilated are D-galactose, D-xylose, L-arabinose, lactose, glycerin, meribiose, libose, D-glucose, D-mannose, maltose, sucrose, trehalose, D-mannitol, starch, raffinose and D-fructose. Growth on a salt-containing Grown at a salt concentration of 12%, but no



growth at a salt medium concentration of 15%.

PGPUB-DOCUMENT-NUMBER: 20020197605

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197605 A1

TITLE: Novel Polynucleotides

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Ando, Seiko	Tokyo		JP	
Hayashi, Mikiro	Tokyo		JP	
Ochiai, Keiko	Tokyo		JP	
Yokoi, Haruhiko	Tokyo		JP	
Tateishi, Naoko	Tokyo		JP	
Senoh, Akihiro	Tokyo		JP	
Ikeda, Masato	Tokyo		JP	
Ozaki, Akio	Hofu-shi		JP	

APPL-NO: 09/ 738626

DATE FILED: December 18, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	P. HEI 11-377484	1999JP-P. HEI 11-377484	December 16, 1999
JP	P. 2000-159162	2000JP-P. 2000-159162	April 7, 2000
JP	P. 2000-280988	2000JP-P. 2000-280988	August 3, 2000

US-CL-CURRENT: 435/6,435/287.2 ,435/91.2

ABSTRACT:

Novel polynucleotides derived from microorganisms belonging to coryneform bacteria and fragments thereof, polypeptides encoded by the polynucleotides and fragments thereof, polynucleotide arrays comprising the polynucleotides and fragments thereof, recording media in which the nucleotide sequences of the polynucleotide and fragments thereof have been recorded which are readable in a computer, and use of them.

----- KWIC -----

Detail Description Table CWU - DETL:

A3(2) 45.0 56.0 95 putative ATP/GTP-binding protein 1347 4847 1281714 1281262  
453 sp:YQJC\_BACSU *Bacillus subtilis* yqjC 35.8 68.7 134 hypothetical protein  
1348 4848 1281794 1282105 312 sp:YC20\_MYCTU *Mycobacterium tuberculosis* 54.5  
79.2 101 hypothetical protein H37Rv Rv1898 1349 4849 1282194 1283114 921  
sp:YD24\_MYCTU *Mycobacterium tuberculosis* 37.9 71.4 301 thioredoxin H37Rv  
Rv1324 1350 4850 1283324 1284466 1143 gp:ECO237695\_3 *Escherichia coli* K12  
ssuD 50.3 74.3 366 FMNH<sub>2</sub>-dependent aliphatic sulfonate monooxygenase 1351  
4851 1284517 1285284 768 sp:SSUC\_ECOLI *Escherichia coli* K12 ssuC 40.8 75.8 240  
aliphatic sulfonates transport permease protein 1352 4852 1285302 1286030  
729 sp:SSUB\_ECOLI *Escherichia coli* K12 ssuB 50.4 72.8 228 aliphatic sulfonates  
transport permease protein 1353 4853 1286043 1286999 957 sp:SSUA\_ECOLI  
*Escherichia coli* K12 ssuA 35.1 62.1 311 sulfonate binding protein precursor  
1354 4854 1289473 1287281 2193 sp:GLGB\_ECOLI *Mycobacterium tuberculosis* 46.1  
72.7 710 1,4-alpha-glucan branching enzyme H37Rv Rv1326c glgB (glycogen  
branching enzyme) 1355 4855 1291007 1289514 1494 sp:AMY3\_DICTH *Dictyoglomus*  
*thermophilum* 22.9 50.5 467 alpha-amylase amyC 1356 4856 1291026 1291373 348  
1357 4857 1291699 1292577 879 sp:FEP\_C\_ECOLI *Escherichia coli* K12 fepC 31.8  
87.6 211 ferric enterobactin transport ATP-binding protein or ABC transport  
ATP-binding protein 1358 4858 1293222 1294025 804 pir:C70860 *Mycobacterium*  
*tuberculosis* 39.6 68.5 260 hypothetical protein H37Rv Rv3040c 1359 4859  
1294151 1295206 1056 pir:H70859 *Mycobacterium tuberculosis* 43.1 70.0 367  
hypothetical protein H37Rv Rv3037c 1360 4860 1295047 1294436 612 1361 4861  
1295435 1296220 786 sp:FIXA\_RHIME *Rhizobium meliloti* fixA 31.2 64.8 244  
electron transfer flavoprotein beta-subunit 1362 4862 1296253 1297203 951  
sp:FIXB\_RHIME *Rhizobium meliloti* fixB 33.1 61.8 335 electron transfer  
flavoprotein alpha-subunit for various dehydrogenases 1363 4863 1296479  
1297093 615 1364 4864 1297212 1298339 1128 sp:NIFS\_AZOVI *Azotobacter*  
*vinelandii* nifS 35.2 67.7 375 nitrogenase cofactor synthesis protein 1365 4865  
1298653 1298342 312 1366 4866 1300145 1299000 1146 sp:Y4ME\_RHISN *Rhizobium* sp.  
NGR234 plasmid 29.5 55.7 397 hypothetical protein pNGR234a y4mE 1367 4867  
1300369 1300145 225 sp:Y4MF\_RHISN *Rhizobium* sp. NGR234 plasmid 47.5 76.3 59  
transcriptional regulator pNGR234a Y4mF 1368 4868 1300552 1301055 504  
sp:YHBS\_ECOLI *Escherichia coli* K12 MG1655 34.8 55.3 181 acetyltransferase  
yhbS 1369 4869 1301929 1300988 942 1370 4870 1303123 1301975 1149 1371 4871  
1303299 1303694 396 1372 4872 1303829 1304923 1095 pir:C70858 *Mycobacterium*  
*tuberculosis* 61.8 80.9 361 tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase 1373 4873 1304536 1303883 654 1374 4874  
1304932 1305921 990 pir:B70857 *Mycobacterium tuberculosis* 33.7 66.0 332  
hypothetical protein H37Rv Rv3015c 1375 4875 1307384 1305924 1461  
sp:TCMA\_STRGA *Streptomyces glaucescens* tcmA 30.2 65.8 500 tetracenomycin C  
resistance and export protein 1376 4876 1308196 1307462 735 1377 4877  
1308330 1310369 2040 sp:DNLJ\_RHOMR *Rhodothermus marinus* dnIJ 42.8 70.6 677 DNA  
ligase (polydeoxyribonucleotide synthase [NAD<sup>+</sup>]) 1378 4878 1311097 1310435  
663 pir:H70856 *Mycobacterium tuberculosis* 40.0 70.9 220 hypothetical protein  
H37Rv Rv3013 1379 4879 1311320 1311616 297 sp:GATC\_STRCO *Streptomyces*  
*coelicolor* A3(2) 53.0 64.0 97 glutamyl-tRNA(Gln) gatC amidotransferase  
subunit C 1380 4880 1311625 1313115 1491 sp:GATA\_MYCTU *Mycobacterium*  
*tuberculosis* 74.0 83.0 484 glutamyl-tRNA(Gln) H37Rv gatA amidotransferase  
subunit A 1381 4881 1313270 1314118 849 sp:VIUB\_VIBVU *Vibrio vulnificus* viuB  
28.1 54.0 263 vibriobactin utilization protein / iron-chelator utilization  
protein 1382 4882 1314775 1314470 306 gp:SCE6\_24 *Streptomyces coelicolor*  
A3(2) 46.9 79.2 96 hypothetical membrane protein SCE6.24 1383 4883 1315013  
1316083 1071 sp:PFP\_AME Amycolatopsis methanolica pfp 54.8 77.9 358

pyrophosphate-fructose 6- phosphate 1-phosphotransferase 1384 4884 1315954  
 1315325 630 1385 4885 1316338 1317444 1107 sp:CCPA\_BACME *Bacillus megaterium*  
 ccpA 31.4 31.4 328 glucose-resistance amylase regulator (catabolite control  
 protein) 1386 4886 1317434 1319005 1572 sp:RBSA\_ECOLI *Escherichia coli* K12  
 rbsA 44.7 76.2 499 ribose transport ATP-binding protein 1387 4887 1319005  
 1319976 972 sp:RBSC\_ECOLI *Escherichia coli* K12 MG1655 45.6 76.9 329 high  
 affinity ribose transport protein rbsC 1388 4888 1320001 1320942 942  
 sp:RBSB\_ECOLI *Escherichia coli* K12 MG1655 45.9 77.7 305 periplasmic  
 ribose-binding protein rbsB 1389 4889 1320952 1321320 369 sp:RBSD\_ECOLI  
*Escherichia coli* K12 MG1655 41.7 68.4 139 high affinity ribose transport  
 protein rbsD 1390 4890 1321476 1322111 636 sp:YIW2\_YEAST *Saccharomyces*  
*cerevisiae* 31.0 58.0 200 hypothetical protein YIR042c 1391 4891 1322393  
 1323406 1014 gp:SCF34\_13 *Streptomyces coelicolor* 31.4 60.2 354  
 iron-siderophore binding lipoprotein SCF34.13c 1392 4892 1323533 1324537  
 1005 sp:NTCI\_RAT *Rattus norvegicus* (Rat) NTCI 35.8 61.9 268 Na-dependent bile  
 acid transporter 1393 4893 1324778 1326256 1479 gsp:W61467 *Staphylococcus*  
*aureus* WHU 29 43.1 71.8 485 RNA-dependent amidotransferase B ratB 1394 4894  
 1326378 1327049 672 sp:F4RE\_METJA *Methanococcus jannaschii* 32.6 61.1 172  
 putative F420-dependent NADH MJ1501 f4re reductase 1395 4895 1330967 1329891  
 1077 sp:YQJG\_ECOLI *Escherichia coli* K12 yqjG 39.8 66.9 317 hypothetical  
 protein 1396 4896 1331102 1331875 774 pir:A70672 *Mycobacterium tuberculosis*  
 39.3 62.4 234 hypothetical protein H37Rv Rv2972c 1397 4897 1331953 1333008  
 1056 pir:H70855 *Mycobacterium tuberculosis* 27.4 52.6 325 hypothetical membrane  
 protein H37Rv Rv3005c 1398 4898 1333424 1333188 237 1399 4899 1335280  
 1333442 1839 gp:AJ012293\_1 *Corynebacterium glutamicum* 99.2 99.4 613  
 dihydroxy-acid dehydratase ATCC 13032 ilvD 1400 4900 1335975 1335412 564  
 pir:G70855 *Mycobacterium tuberculosis* 33.3 68.6 105 hypothetical protein  
 H37Rv Rv3004 1401 4901 1337567 1336095 1473 sp:YILV\_CORGL *Corynebacterium*  
*glutamicum* 100.0 100.0 62 hypothetical membrane protein ATCC 13032 yilV 1402  
 4902 1338609 1338379 231 GP:SSU18930\_263 ***Sulfolobus*** solfataricus 45.0 55.0 66  
 hypothetical protein 1403 4903 1342072 1342677 606 1404 4904 1342457 1341960  
 498 sp:NRTD\_SYNPF *Synechococcus* sp. nrtD 50.9 80.8 167 nitrate transport  
 ATP-binding protein 1405 4905 1342727 1342461 267 sp:MALK\_ENTAE *Enterobacter*  
*aerogenes* 46.0 78.2 87 maltose/maltodextrin transport ATP- (*Aerobacter*  
*aerogenes*) malk binding protein 1406 4906 1343675 1342794 882 sp:NRTA\_ANASP  
*Anabaena* sp strain PCC 7120 28.1 56.8 324 nitrate transporter protein nrtA  
 1407 4907 1344018 1344464 447 1408 4908 1344440 1344808 369 1409 4909  
 1344935 1345420 486 sp:DIM6\_STRCO *Streptomyces coelicolor* 39.4 73.2 142  
 actinorhodin polyketide dimerase 1410 4910 1345486 1346439 954 sp:CZCD\_ALCEU  
*Ralstonia eutropha* czcD 39.1 72.7 304 cobalt-zinc-cadmium resistance protein  
 1411 4911 1345487 1345335 153 1412 4912 1346331 1345642 690 1413 4913 1346458  
 1348272 1815 sp:Y686\_METJA *Methanococcus jannaschii* 22.9 53.7 642 hypothetical  
 protein 1414 4914 1348334 1350076 1743 1415 4915 1350855 1352444 1590  
 gsp:Y22646 *Brevibacterium flavum* serA 99.8 100.0 530 D-3-phosphoglycerate  
 dehydrogenase 1416 4916 1352053 1351727 327 SP:YEN1\_SCHPO *Schizosaccharomyces*  
*pombe* 29.0 52.0 105 hypothetical serine-rich protein SPAC11G7.01 1417 4917  
 1352585 1353451 867 1418 4918 1355601 1354540 1062 1419 4919 1355689 1357554  
 1866 pir:T03476 *Rhodobacter capsulatus* strain 32.9 63.1 620 hypothetical  
 protein SB1003 1420 4920 1356452 1356853 402 1421 4921 1357557 1358210 654  
 1422 4922 1358259 1359062 804 sp:HPCE\_ECOLI *Escherichia coli* C hpcE 33.3 59.2  
 228 homoprotocatechuate catabolism bifunctional isomerase/decarboxylase  
 [includes: 2-hydroxyhepta-2,4-diene-1,7-dioate isomerase(hhdd isomerase); 5-  
 carboxymethyl-2-oxo-hex-3-ene-1,7- dioate decarboxylase(opet decarboxylase)]

1423 4923 1359052 1359669 618 sp:UBIG\_ECOLI Escherichia coli K12 23.4 55.7 192  
 methyltransferase or 3- demethylubiquinone-9 3-O- methyltransferase 1424  
 4924 1361295 1360168 1128 sp:DHBC\_BACSU Bacillus subtilis dhbC 38.0 70.4 371  
 isochorismate synthase 1425 4925 1361361 1362848 1488 sp:SYE\_BACSU Bacillus  
 subtilis gltX 37.3 69.7 485 glutamyl-tRNA synthetase 1426 4926 1363138  
 1362926 213 gp:SCJ33\_10 Streptomyces coelicolor A3(2) 77.0 90.0 67  
 transcriptional regulator 1427 4927 1363657 1363142 516 1428 4928 1364253  
 1363732 522 1429 4929 1364915 1365256 342 1430 4930 1364960 1364340 621 1431  
 4931 1365180 1364878 303

#### Detail Description Table CWU - DETL:

sp:RIMM\_MYCLE Mycobacterium leprae 52.3 72.1 172 16S rRNA processing protein  
 MLCB250.34. rimM 2250 5750 2164390 2164737 348 pir:B71881 Helicobacter pylori  
 J99 jhp0839 29.0 66.7 69 hp0thetical protein 2251 5751 2165309 2164815 495  
 pir:C47154 Bacillus subtilis 168 rpsP 47.0 79.5 83 30S ribosomal protein S16  
 2252 5752 2165523 2166098 576 pir:T14151 Mus musculus inv 32.1 61.7 196  
 inversin 2253 5753 2166990 2166124 867 prf:2512328G Streptococcus agalactiae  
 cylB 26.6 69.1 256 ABC transporter 2254 5754 2167865 2166990 876  
 prf:2220349C Pyrococcus horikoshii OT3 mtrA 35.5 63.8 318 ABC transporter 2255  
 5755 2169584 2167944 1641 sp:SR54\_BACSU Bacillus subtilis 168 ffh 58.7 78.2  
 559 signal recognition particle protein 2256 5756 2170426 2171058 633 2257  
 5757 2171715 2172131 417 2258 5758 2172209 2172877 669 2259 5759 2175288  
 2173759 1530 sp:FTSY\_ECOLI Escherichia coli K12 ftsY 37.0 66.1 505 cell  
 division protein 2260 5760 2176046 2175888 159 2261 5761 2176402 2177103 702  
 2262 5762 2179502 2176110 3393 sp:AMYLH\_YEAST Saccharomyces cerevisiae 22.4  
 46.2 1144 glucan 1,4-alpha-glucosidase or S288C YIR019C sta1 glucoamylase  
 S1/S2 precursor 2263 5763 2180918 2181880 963 2264 5764 2183092 2179628 3465  
 sp:Y06B\_MYCTU Mycobacterium tuberculosis 48.3 72.6 1206 chromosome segregation  
 protein H37Rv Rv2922c smc 2265 5765 2183391 2183110 282 sp:ACYP\_MYCTU  
 Mycobacterium tuberculosis 51.1 73.9 92 acylphosphatase H37Rv RV2922.1C 2266  
 5766 2185258 2183405 1854 2267 5767 2186208 2185351 858 sp:YFER\_ECOLI  
 Escherichia coli K12 yfeR 23.9 60.0 305 transcriptional regulator 2268 5768  
 2186299 2187129 831 pir:S72748 Mycobacterium leprae 39.3 73.5 257 hypothetical  
 membrane protein MLCL581.28c 2269 5769 2187160 2187342 183 2270 5770 2187679  
 2187233 447 2271 5771 2188306 2187692 615 gp:DNINTREG\_3 Dichelobacter nodosus  
 gep 46.8 76.6 188 cation efflux system protein 2272 5772 2189170 2188313 858  
 sp:FPG\_ECOLI Escherichia coli K12 mutM or 36.1 66.7 285  
 formamidopyrimidine-DNA fpg glycosylase 2273 5773 2189906 2189166 741  
 pir:B69693 Bacillus subtilis 168 rncS 40.3 76.5 221 ribonuclease III 2274  
 5774 2190439 2189906 534 sp:Y06F\_MYCTU Mycobacterium tuberculosis 35.8 62.5  
 176 hypothetical protein H37Rv Rv2926c 2275 5775 2191328 2190540 789  
 sp:Y06G\_MYCTU Mycobacterium tuberculosis 50.0 76.9 238 hypothetical protein  
 H37Rv Rv2927c 2276 5776 2191522 2193165 1644 prf:2104260G Streptomyces  
 verticillus 28.3 55.6 559 transport protein 2277 5777 2193165 2194694 1530  
 sp:CYDC\_ECOLI Escherichia coli K12 cydC 26.6 58.8 541 ABC transporter 2278  
 5778 2196883 2198004 1122 gp:SC9C7\_2 Streptomyces coelicolor A3(2) 35.3 62.6  
 388 hypothetical protein SC9C7.02 2279 5779 2198447 2198007 441 2280 5780  
 2198475 2199758 1284 pir:A72322 Thermotoga maritima MSB8 21.0 43.7 405  
 hypothetical protein TM0896 2281 5781 2199808 2201070 1263 sp:HIPO\_CAMJE  
 Campylobacter jejuni ATCC 32.9 64.3 353 peptidase 43431 hipO 2282 5782  
 2201408 2201073 336 pir:S38197 Arabidopsis thaliana SUC1 27.1 51.9 133 sucrose

transport protein 2283 5783 2201584 2201450 135 2284 5784 2201869 2201594  
 276 2285 5785 2204541 2201992 2550 prf:2513410A *Thermococcus litoralis* malP  
 36.1 67.4 814 maltodextrin phosphorylase / glycogen phosphorylase 2286 5786  
 2205490 2204591 900 sp:YFIE\_BACSU *Bacillus subtilis* 168 yfiE 33.9 66.4 295  
 hypothetical protein 2287 5787 2208249 2207302 948 sp:LGT\_STAAU  
*Staphylococcus aureus* FDA 485 31.4 65.5 264 prolipoprotein diacylglycerol lgt  
 transferase 2288 5788 2209167 2208367 801 sp:TRPG\_EMENI *Emericella nidulans*  
 trpC 29.6 62.1 169 indole-3-glycerol-phosphate synthase/anthranilate synthase  
 component II 2289 5789 2209888 2209232 657 pir:H70556 *Mycobacterium*  
*tuberculosis* 29.4 58.8 228 hypothetical membrane protein H37Rv Rv1610 2290  
 5790 2210273 2209920 354 sp:HIS3\_RHOSH *Rhodobacter sphaeroides* ATCC 52.8 79.8  
 89 phosphoribosyl-AMP cyclohydrolase 17023 hisI 2291 5791 2211046 2210273  
 774 sp:HIS6\_CORG *Corynebacterium glutamicum* 97.3 97.7 258 cyclase AS019 hisF  
 2292 5792 2211875 2211051 825 prf:2419176B *Corynebacterium glutamicum* 94.0  
 94.0 241 inositol monophosphate AS019 impA phosphatase 2293 5793 2212619  
 2211882 738 gp:AF051846\_1 *Corynebacterium glutamicum* 95.9 97.6 245  
 phosphoribosylformimino-5- AS019 hisA aminoimidazole carboxamide ribotide  
 isomerase 2294 5794 2213273 2212641 633 gp:AF060558\_1 *Corynebacterium*  
*glutamicum* 86.7 92.4 210 glutamine amidotransferase AS019 hisH 2295 5795  
 2215586 2214321 1266 sp:CMLR\_STRLI *Streptomyces lividans* 66 cmIR 25.6 54.0 402  
 chloramphenicol resistance protein or transmembrane transport protein 2296  
 5796 2215863 2215639 225 2297 5797 2216474 2215869 606 sp:HIS7\_STRCO  
*Streptomyces coelicolor* A3(2) 52.5 81.8 198 imidazoleglycerol-phosphate hisB  
 dehydratase 2298 5798 2217591 2216494 1098 sp:HIS8\_STRCO *Streptomyces*  
*coelicolor* A3(2) 57.2 79.3 362 histidinol-phosphate hisC aminotransferase  
 2299 5799 2218925 2217600 1326 sp:HISX\_MYCSM *Mycobacterium smegmatis* 63.8 85.7  
 439 histidinol dehydrogenase ATCC 607 hisD 2300 5800 2219159 2220358 1200  
 gp:SPBC215\_13 *Schizosaccharomyces pombe* 27.2 54.4 342 serine-rich secreted  
 protein SPBC215.13 2301 5801 2221109 2220459 651 2302 5802 2221611 2221919  
 309 2303 5803 2221828 2221187 642 prf:2321269A *Leishmania donovani* SACP-1  
 29.4 59.7 211 histidine secretory acid phosphatase 2304 5804 2221958 2222518  
 561 pir:RPECR1 *Escherichia coli* plasmid RP1 28.9 60.8 204 tet repressor  
 protein tetR 2305 5805 2222528 2225035 2508 prf:2307203B **Sulfolobus**  
**acidocaldarius** treX 47.4 75.5 722 glycogen debranching enzyme 2306 5806  
 2225149 2225949 801 pir:E70572 *Mycobacterium tuberculosis* 50.0 76.0 258  
 hypothetical protein H37Rv Rv2622 2307 5807 2226763 2225990 774 gp:SC2G5\_27  
*Streptomyces coelicolor* A3(2) 29.9 55.2 268 oxidoreductase SC2G5.27c gip 2308  
 5808 2227779 2226769 1011 prf:2503399A *Sinorhizobium meliloti* idhA 35.0 60.9  
 343 myo-inositol 2-dehydrogenase 2309 5809 2227906 2228901 996 sp:GALR\_ECOLI  
*Escherichia coli* K12 galR 30.4 64.4 329 galactitol utilization operon  
 repressor 2310 5810 2229896 2229099 798 sp:FHUC\_BACSU *Bacillus subtilis* 168  
 fhuC 32.9 68.3 246 ferrichrome transport ATP-binding protein or ferrichrome  
 ABC transporter 2311 5811 2230937 2229900 1038 prf:2423441E *Vibrio cholerae*  
 hutC 36.8 71.1 332 hemin permease 2312 5812 2231294 2230947 348 pir:G70046  
*Bacillus subtilis* 168 yvrC 30.1 68.0 103 iron-binding protein 2313 5813  
 2231932 2231339 594 pir:G70046 *Bacillus subtilis* 168 yvrC 34.6 67.6 182  
 iron-binding protein 2314 5814 2232456 2232016 441 sp:YTFH\_ECOLI *Escherichia*  
*coli* K12 ytfH 38.1 73.5 113 hypothetical protein 2315 5815 2232928 2234070  
 1143 gp:SCI8\_12 *Streptomyces coelicolor* A3(2) 23.4 50.1 355 DNA polymerase III  
 epsilon chain SCI8.12 2316 5816 2234158 2234763 606 2317 5817 2234852  
 2237284 2433 pir:S65769 *Arthrobacter* sp. Q36 treY 42.0 68.6 814 maltooligosyl  
**trehalose** synthase 2318 5818 2237331 2238353 1023 gp:AE002006\_4 *Deinococcus*  
*radiodurans* 27.6 52.8 322 hypothetical protein DR1631 2319 5819 2239092

2238694 399 2320 5820 2240042 2239845 198 2321 5821 2240246 2240058 189  
 2322 5822 2240563 2239508 1056 2323 5823 2240681 2241724 1044 sp:LXA1\_PHOLU  
 Photorhabdus luminescens 20.5 54.4 375 alkanal monooxygenase alpha chain ATCC  
 29999 luxA 2324 5824 2242115 2241738 378 gp:SC7H2\_5 Streptomyces coelicolor  
 A3(2) 58.3 79.2 120 hypothetical protein SC7H2.05 2325 5825 2242359 2242129  
 231 2326 5826 2243035 2244819 1785 pir:S65770 Arthrobacter sp. Q36 treZ 46.3  
 72.4 568 maltotrioligosyltrehalose trehalohydrolase 2327 5827 2243043 2242393  
 651 sp:YVYE\_BACSU Bacillus subtilis 168 36.5 72.4 214 hypothetical protein  
 2328 5828 2246171 2244864 1308 sp:THD1\_CORGL Corynebacterium glutamicum 99.3  
 99.3 436 threonine dehydratase ATCC 13032 ilvA 2329 5829 2246386 2246892  
 507 2330 5830 2246450 2246295 156 2331 5831 2248208 2247006 1203 pir:S57636  
 Catharanthus roseus metE 22.7 49.6 415 Corynebacterium glutamicum ASO19 2332  
 5832 2251939 2248358 3582 prf:2508371A Streptomyces coelicolor A3(2) 53.3 80.5  
 1183 DNA polymerase III dnaE 2333 5833 2252017 2252856 840 sp:RARD\_ECOLI  
 Escherichia coli K12 rarD 37.6 73.8 279 chloramphenicol sensitive protein  
 2334 5834 2253192 2253659 468 sp:HISJ\_CAMJE Campylobacter jejuni DZ72 hisJ  
 21.5 55.7 149 histidine-binding protein precursor 2335 5835 2253725 2254642  
 918 pir:D69548 Archaeoglobus fulgidus AF2388 22.7 64.7 198 hypothetical  
 membrane protein 2336 5836 2255558 2254683 876 sp:GS39\_BACSU Bacillus  
 subtilis 168 ydaD 48.2 80.0 280 short chain dehydrogenase or general stress  
 protein 2337 5837 2257024 2255738 1287 sp:DCDA\_PSEAE Pseudomonas aeruginosa  
 lysA 22.9 47.6 445 diaminopimelate (DAP) decarboxylase 2338 5838 2259312  
 2258362 951 sp:CYSM\_ALCEU Alcaligenes eutrophus CH34 32.8 64.3 314 cysteine  
 synthase cysM 2339 5839 2259999 2259421 579 2340 5840 2260931 2260002 930  
 sp:RLUD\_ECOLI Escherichia coli K12 rluD 36.5 61.0 326 ribosomal large subunit  
 pseudouridine synthase D 2341 5841 2261467 2260934 534 sp:LSPA\_PSEFL  
 Pseudomonas fluorescens NCIB 33.8 61.7 154 lipoprotein signal peptidase 10586  
 lspA 2342 5842 2261688 2262689 1002

#### Detail Description Table CWU - DETL:

H37Rv Rv2345 2496 5996 2410264 2409779 486 gp:AE003565\_26 Drosophila  
 melanogaster 24.6 54.4 138 hypothetical protein CG10592 2497 5997 2410861  
 2410280 582 2498 5998 2412338 2410956 1383 pir:S58522 Thermus aquaticus HB8  
 46.1 69.9 508 glycyl-tRNA synthetase 2499 5999 2412580 2412948 369 pir:E70585  
 Mycobacterium tuberculosis 49.4 73.0 89 bacterial regulatory protein, arsR  
 H37Rv Rv2358 furB family, 2500 6000 2412992 2413423 432 sp:FUR\_ECOLI  
 Escherichia coli K12 fur 34.9 70.5 132 ferric uptake regulation protein 2501  
 6001 2413568 2415118 1551 pir:A70539 Mycobacterium tuberculosis 24.8 46.7 529  
 hypothetical protein (conserved in H37Rv Rv1128c C.glutamicum?) 2502 6002  
 2416089 2415298 792 gp:AF162938\_1 Streptomyces coelicolor A3(2) 40.6 67.0 224  
 hypothetical membrane protein h3u 2503 6003 2417099 2416371 729  
 sp:UPPS\_MICLU Micrococcus luteus B-P 26 uppS 43.4 71.2 233 undecaprenyl  
 diphosphate synthase 2504 6004 2417947 2417222 726 pir:A70586 Mycobacterium  
 tuberculosis 45.7 74.3 245 hypothetical protein H37Rv Rv2362c 2505 6005  
 2418883 2417969 915 gp:AF072811\_1 Streptococcus pneumoniae era 39.5 70.3 296  
 Era-like GTP-binding protein 2506 6006 2420309 2418990 1320 sp:Y1DE\_MYCTU  
 Mycobacterium tuberculosis 52.8 82.4 432 hypothetical membrane protein H37Rv  
 Rv2366 2507 6007 2420900 2420313 588 sp:YN67\_MYCTU Mycobacterium tuberculosis  
 65.0 86.0 157 hypothetical protein H37Rv Rv2367c 2508 6008 2420973 2421236  
 264 GSP:Y75650 Neisseria meningitidis 45.0 50.0 85 Neisserial polypeptides  
 predicted to be useful antigens for vaccines and diagnostics 2509 6009

2421949 2420900 1050 sp:PHOL\_MYCTU Mycobacterium tuberculosis 61.1 84.6 344  
 phosphate starvation inducible H37Rv Rv2368c phoH protein 2510 6010 2422697  
 2421975 723 gp:SCC77\_19 Streptomyces coelicolor A3(2) 44.0 75.4 248  
 hypothetical protein SCC77.19c. 2511 6011 2422850 2423791 942 2512 6012  
 2423845 2422700 1146 prf:2421342B Streptomyces albus dnaJ2 47.1 77.4 380 heat  
 shock protein dnaJ 2513 6013 2424937 2423915 1023 prf:2421342A Streptomyces  
 albus hrcA 48.2 79.6 334 heat-inducible transcriptional repressor (groEL  
 repressor) 2514 6014 2425954 2424965 990 prf:2318256A Bacillus  
 stearothermophilus 33.1 64.1 320 oxygen-independent hemN coproporphyrinogen  
 III oxidase 2515 6015 2426181 2426699 519 sp:AGA1\_YEAST Saccharomyces  
 cerevisiae 36.6 64.9 134 agglutinin attachment subunit YNR044WAGA1 precursor  
 2516 6016 2427468 2426776 693 2517 6017 2428184 2427807 378 2518 6018  
 2430028 2428184 1845 gp:SC6G10\_4 Streptomyces coelicolor A3(2) 48.0 75.1 611  
 long-chain-fatty-acid-CoA ligase SC6G10.04 2519 6019 2430296 2432413 2118  
 sp:MALQ\_ECOLI Escherichia coli K12 malQ 28.3 55.4 738  
 4-alpha-glucanotransferase 2520 6020 2432508 2434370 1863 gp:AB005752\_1  
 Lactobacillus brevis plasmid 29.5 64.4 604 ABC transporter, Hop-Resistance  
 horA protein 2521 6021 2433868 2433614 255 GSP:Y74827 Neisseria gonorrhoeae  
 44.0 51.0 68 Neisserial polypeptides predicted to be useful antigens for  
 vaccines and diagnostics 2522 6022 2434207 2433875 333 GSP:Y74829 Neisseria  
 meningitidis 47.0 53.0 107 polypeptides predicted to be useful antigens for  
 vaccines and diagnostics 2523 6023 2434619 2434440 180 2524 6024 2434776  
 2434573 204 2525 6025 2436838 2434805 2034 sp:DCP\_SALTY Salmonella  
 typhimurium dcp 40.3 68.3 690 peptidyl-dipeptidase 2526 6026 2436871 2438049  
 1179 gp:AF064523\_1 Anisopteromalus calandrae 24.1 45.7 453 carboxylesterase  
 2527 6027 2438113 2439906 1794 pir:G70983 Mycobacterium tuberculosis 65.2 84.9  
 594 glycosyl hydrolase or trehalose H37Rv Rv0126 synthase 2528 6028 2439906  
 2440994 1089 pir:H70983 Mycobacterium tuberculosis 32.1 58.8 449 hypothetical  
 protein H37Rv Rv0127 2529 6029 2441589 2441005 585 pir:T07979 Chlamydomonas  
 reinhardtii ipi1 31.8 57.7 189 isopentenyl-diphosphate Delta- isomerase 2530  
 6030 2441669 2441890 222 2531 6031 2442355 2442792 438 2532 6032 2443356  
 2441602 1755 2533 6033 2444015 2443356 660 2534 6034 2444551 2444033 519  
 2535 6035 2444735 2445709 975 gp:CORCSLYS\_1 Corynebacterium glutamicum 99.4  
 100.0 325 beta C-S lyase (degradation of ATCC 13032 aecD aminoethylcysteine)  
 2536 6036 2445716 2446993 1278 sp:BRNQ\_CORGL Corynebacterium glutamicum 99.8  
 100.0 426 branched-chain amino acid transport ATCC 13032 brnQ system carrier  
 protein (isoleucine uptake) 2537 6037 2447021 2447998 978 sp:LUXA\_VIBHA  
 Vibrio harveyi luxA 21.6 49.0 343 alkanal monooxygenase alpha chain 2538 6038  
 2450844 2450323 522 2539 6039 2451785 2450859 927 gp:AF155772\_2  
 SinoRhizobium meliloti mdcF 25.9 60.5 324 malonate transporter 2540 6040  
 2454637 2451794 2844 sp:GLCD\_ECOLI Escherichia coli K12 glcD 27.7 55.1 483  
 glycolate oxidase subunit 2541 6041 2454725 2455435 711 sp:YDFH\_ECOLI  
 Escherichia coli K12 ydfH 25.6 65.0 203 transcriptional regulator 2542 6042  
 2455733 2455452 282 2543 6043 2457066 2455720 1347 sp:YGIK\_SALTY Salmonella  
 typhimurium ygiK 22.5 57.6 467 hypothetical protein 2544 6044 2457759 2457337  
 423 2545 6045 2457863 2459371 1509 sp:HBP\_A\_HAEIN Haemophilus influenzae Rd  
 27.5 55.5 546 heme-binding protein A precursor HI0853 hbpA (hemin-binding  
 lipoprotein) 2546 6046 2459371 2460336 966 sp:APPB\_BACSU Bacillus subtilis  
 168 appB 40.0 73.3 315 oligopeptide ABC transporter (permease) 2547 6047  
 2460340 2461167 828 sp:DPPC\_ECOLI Escherichia coli K12 dppC 43.2 74.5 271  
 dipeptide transport system permease protein 2548 6048 2461163 2462599 1437  
 prf:2306258MR Escherichia coli K12 oppD 37.4 66.4 372 oligopeptide transport  
 ATP-binding protein 2549 6049 2462049 2461543 507 PIR:G72536 Aeropyrum pernix



K1 APE1580 35.0 44.0 106 hypothetical protein 2550 6050 2463150 2462602 549  
 pir:D70367 Aquifex aeolicus VF5 aq\_768 29.3 58.0 157 hypothetical protein 2551  
 6051 2463241 2464143 903 prf:2514301A Rhizobium etli rbsK 41.0 65.0 300 ribose  
 kinase 2552 6052 2464344 2465768 1425 gp:SCM2\_16 Streptomyces coelicolor  
 A3(2) 39.9 64.6 466 hypothetical membrane protein SCM2.16c 2553 6053 2465767  
 2465465 303 2554 6054 2467009 2466038 972 sp:NTCI\_HUMAN Homo sapiens 31.3  
 61.6 284 sodium-dependent transporter or sodium Bile acid symporter family  
 2555 6055 2467077 2467922 846 gp:AF195243\_1 Chlamydomonas reinhardtii 28.5  
 51.2 295 apospory-associated protein C 2556 6056 2470313 2470678 366 2557  
 6057 2472250 2472819 570 sp:THIX\_CORGL Corynebacterium glutamicum 100.0 100.0  
 133 thiamine biosynthesis protein X ATCC 13032 thiX 2558 6058 2473480  
 2472893 588 sp:VG66\_BPMD Mycobacteriophage D29 66 42.6 65.5 197 hypothetical  
 protein 2559 6059 2473653 2475542 1890 sp:BETP\_CORGL Corynebacterium  
 glutamicum 39.8 71.7 601 glycine betaine transporter ATCC 13032 betP 2560  
 6060 2476497 2477492 996 2561 6061 2477644 2479251 1608 2562 6062 2479379  
 2479762 384

#### Detail Description Table CWU - DETL:

624 gp:AF071885\_2 Streptomyces coelicolor M145 69.5 88.3 197 ATP-dependent Clp  
 protease clpP2 proteolytic subunit 2 2648 6148 2556580 2555978 603  
 gp:AF071885\_1 Streptomyces coelicolor M145 62.1 85.9 198 ATP-dependent Clp  
 protease clpP1 proteolytic subunit 1 2649 6149 2556599 2556748 150  
 gp:SIS243537\_4 **Sulfolobus** islandicus ORF154 42.9 71.4 42 hypothetical protein  
 2650 6150 2558106 2556760 1347 sp:TIG\_BACSU Bacillus subtilis 168 tig 32.1  
 66.4 417 trigger factor (prolyl isomerase) (chaperone protein) 2651 6151  
 2558609 2559103 495 gp:SCD25\_17 Streptomyces coelicolor A3(2) 32.5 63.1 160  
 hypothetical protein SCD25.17 2652 6152 2559157 2560131 975 sp:PBP4\_NOCLA  
 Nocardia lactamdurans LC411 25.3 50.9 336 penicillin-binding protein pbp  
 2653 6153 2560131 2560586 456 prf:2301342A Mus musculus Moa1 27.8 58.3 115  
 hypothetical protein 2654 6154 2561115 2561363 249 2655 6155 2561920 2561483  
 438 prf:2513302C Corynebacterium striatum ORF1 54.2 73.2 142 transposase 2656  
 6156 2562093 2562242 150 2657 6157 2562115 2561990 126 prf:2513302C  
 Corynebacterium striatum ORF1 57.1 82.9 35 hypothetical protein 2658 6158  
 2562341 2562078 264 prf:2513302C Corynebacterium striatum ORF1 50.7 78.7 75  
 transposase 2659 6159 2562776 2562387 390 2660 6160 2562963 2563847 885 2661  
 6161 2564402 2563932 471 sp:LACB\_STAAU Staphylococcus aureus NCTC 40.0 71.4  
 140 galactose-6-phosphate isomerase 8325-4 lacB 2662 6162 2565245 2564550 696  
 sp:YAMY\_BACAD Bacillus acidopullulyticus ORF2 26.2 58.1 248 hypothetical  
 protein 2663 6163 2566231 2565623 609 pir:A70866 Mycobacterium tuberculosis  
 56.8 80.9 199 hypothetical protein H37Rv Rv2466c 2664 6164 2566345 2568945  
 2601 sp:AMPN\_STRLI Streptomyces lividans pepN 47.5 70.5 890 aminopeptidase N  
 2665 6165 2569211 2570293 1083 pir:B70206 Borrelia burgdorferi BB0852 25.1  
 58.1 358 hypothetical protein 2666 6166 2571460 2570309 1152 2667 6167  
 2571510 2572175 666 2668 6168 2572193 2572348 156 2669 6169 2572677 2572351  
 327 gp:AF139916\_3 Brevibacterium linens ATCC 61.5 81.7 104 phytoene  
 desaturase 9175 crtI 2670 6170 2572977 2572807 171 2671 6171 2573770 2573393  
 378 2672 6172 2573864 2572659 1206 sp:CRTJ\_MYXXA Myxococcus xanthus DK1050  
 31.2 63.8 381 phytoene dehydrogenase carA2 2673 6173 2574718 2573843 876  
 sp:CRTB\_STRGR Streptomyces griseus JA3933 31.4 58.6 290 phytoene synthase crtB  
 2674 6174 2575898 2574780 1119 gp:LMAJ9627\_3 Listeria monocytogenes lftB 25.8  
 47.7 392 multidrug resistance transporter 2675 6175 2577213 2575981 1233

2676 6176 2578872 2577232 1641 gp:SYOATPBP\_2 *Synechococcus elongatus* 41.3 71.6  
 538 ABC transporter ATP-binding protein 2677 6177 2579760 2578879 882  
 sp:DPPC\_BACFI *Bacillus firmus* OF4 dppC 38.8 73.8 286 dipeptide transport  
 system permease protein 2678 6178 2580707 2579769 939 pir:S47696 *Escherichia*  
*coli* K12 nikB 33.2 62.0 316 nickel transport system permease protein 2679  
 6179 2582417 2580711 1707 2680 6180 2582564 2584504 1941 2681 6181 2584613  
 2585926 1314 sp:ARGD\_CORGL *Corynebacterium glutamicum* 31.4 63.5 411  
 acetylornithine aminotransferase ATCC 13032 argD 2682 6182 2586180 2587763  
 1584 pir:A70539 *Mycobacterium tuberculosis* 25.1 47.9 482 hypothetical protein  
 H37Rv Rv1128c 2683 6183 2587976 2588722 747 sp:YA26\_MYCTU *Mycobacterium*  
*tuberculosis* 49.1 79.4 218 hypothetical membrane protein H37Rv Rv0364 2684  
 6184 2589432 2588725 708 sp:PHBB\_CHRVI *Chromatium vinosum* D phbB 28.1 60.0 235  
 acetoacetyl CoA reductase 2685 6185 2589565 2590302 738 pir:A40046  
*Streptomyces coelicolor* actII 26.7 55.0 240 transcriptional regulator, TetR  
 family 2686 6186 2590697 2591137 441 GSP:Y74375 *Neisseria meningitidis* 38.0  
 47.0 94 polypeptides predicted to be useful antigens for vaccines and  
 diagnostics 2687 6187 2592365 2591574 792 gp:AF106002\_1 *Pseudomonas putida*  
 GM73 31.1 65.1 238 ABC transporter ATP-binding protein ttg2A 2688 6188  
 2592402 2592794 393 gp:MLCB1610\_9 *Mycobacterium leprae* 53.2 77.0 126 globin  
 MLCB1610.14c 2689 6189 2592838 2593965 1128 sp:CHRA\_PSEAE *Pseudomonas*  
*aeruginosa* 27.3 60.4 396 chromate transport protein Plasmid pUM505 chrA 2690  
 6190 2594594 2593968 627 pir:A70867 *Mycobacterium tuberculosis* 37.8 68.9 196  
 hypothetical protein H37Rv Rv2474c 2691 6191 2595061 2594597 465  
 gp:SC6D10\_19 *Streptomyces coelicolor* A3(2) 36.2 61.4 127 hypothetical protein  
 SC6D10.19c 2692 6192 2595808 2595188 621 2693 6193 2595983 2595822 162  
 pir:B72589 *Aeropyrum pernix* K1 APE1182 36.4 60.0 55 hypothetical protein 2694  
 6194 2597715 2596048 1668 sp:YJJK\_ECOLI *Escherichia coli* K12 yjK 52.8 79.6  
 563 ABC transporter ATP-binding protein 2695 6195 2598483 2597869 615  
 pir:E70867 *Mycobacterium tuberculosis* 31.4 62.2 172 hypothetical protein  
 H37Rv Rv2478c 2696 6196 2600764 2598662 2103 sp:Y05L\_MYCLE *Mycobacterium*  
*leprae* 0659 28.0 56.7 700 hypothetical membrane protein 2697 6197 2601461  
 2602879 1419 pir:C69676 *Bacillus subtilis* phoB 28.0 52.6 536 alkaline  
 phosphatase 2698 6198 2604573 2605502 930 2699 6199 2604583 2603945 639  
 2700 6200 2605520 2604609 912 sp:MSMG\_STRMU *Streptococcus mutans* 39.1 76.3 279  
 multiple sugar-binding transport INGBRITTmsmG system permease protein 2701  
 6201 2606369 2605527 843 sp:MSMF\_STRMU *Streptococcus mutans* 27.4 67.5 292  
 multiple sugar-binding transport INGBRITTmsmF system permease protein 2702  
 6202 2606444 2608117 1674 2703 6203 2607889 2606561 1329 prf:2206392C  
*Thermoanaerobacterium* 28.8 63.2 462 maltose-binding protein thermosul amyE  
 2704 6204 2609426 2608185 1242 2705 6205 2610639 2609512 1128 prf:2308356A  
*Streptomyces reticuli* msiK 59.1 79.8 386 ABC transporter ATP-binding protein  
 (ABC-type sugar transport protein) or cellobiose/maltose transport protein  
 2706 6206 2611523 2612272 750 2707 6207 2611531 2610848 684 prf:2317468A  
*Schizosaccharomyces pombe* 37.7 72.7 154 dolichol phosphate mannose dpm1  
 synthase 2708 6208 2612462 2613151 690 2709 6209 2613712 2614500 789  
 prf:2516398E *Rhodococcus rhodochrous* 67.2 89.4 207 aldehyde dehydrogenase  
 plasmid pRTL1 orf5 2710 6210 2614649 2615410 762 prf:2513418A *Synechococcus*  
 sp. PCC7942 48.6 73.8 183 circadian phase modifier cpmA 2711 6211 2615451  
 2615795 345 2712 6212 2617120 2615939 1182 pir:A72312 *Thermotoga maritima*  
 MSB8 35.0 64.6 412 hypothetical membrane protein TM0964 2713 6213 2617246  
 2617995 750 sp:GIP\_ECOLI *Escherichia coli* K12 gip 41.2 69.4 255  
 glyoxylate-induced protein 2714 6214 2618072 2618869 798 pir:E70761  
*Mycobacterium tuberculosis* 40.0 57.0 258 ketoacyl reductase H37Rv Rv1544 2715

6215 2618882 2619538 657 sp:ORN\_ECOLI Escherichia coli K12 orn 48.0 78.8 179  
oligoribonuclease 2716 6216 2620728 2619541 1188 prf:2409378A Salmonella  
enterica iroD 26.0 50.9 454 ferric enterochelin esterase 2717 6217 2622181  
2620973 1209

#### Detail Description Table CWU - DETL:

protein 2811 6311 2713702 2713842 141 PIR:F81737 Chlamydia muridarum Nigg  
71.0 75.0 42 hypothetical protein TC0129 2812 6312 2718187 2717993 195 2813  
6313 2719689 2718436 1254 sp:MURA\_ACICA Acinetobacter calcoaceticus 44.8 75.3  
417 UDP-N-acetylglucosamine 1- NCIB 8250 murA carboxyvinyltransferase 2814  
6314 2719750 2720319 570 sp:Y02Y\_MYCTU Mycobacterium tuberculosis 66.3 84.2  
190 hypothetical protein H37Rv Rv1314c 2815 6315 2721227 2720385 843  
gp:SC2G5\_15 Streptomyces coelicolor A3(2) 45.9 69.0 281 transcriptional  
regulator SC2G5.15c 2816 6316 2721702 2721295 408 2817 6317 2721934 2722857  
924 sp:CYSK\_BACSU Bacillus subtilis 168 cysK 57.1 84.6 305 cysteine synthase  
2818 6318 2723064 2723609 546 prf:2417357C Azotobacter vinelandii cysE2 61.1  
79.7 172 O-acetylserine synthase 2819 6319 2724057 2723770 288 gp:AE002024\_10  
Deinococcus radiodurans R1 36.1 65.1 83 hypothetical protein DR1844 2820  
6320 2725359 2724478 882 sp:SUCD\_COXBU Coxiella burnetii Nine Mile Ph I 52.9  
79.4 291 succinyl-CoA synthetase alpha sucD chain 2821 6321 2725619 2725843  
225 PIR:F72706 Aeropyrum pernix K1 APE1069 42.0 43.0 75 hypothetical protein  
2822 6322 2726577 2725384 1194 sp:SUCB\_BACSU Bacillus subtilis 168 sucC 39.8  
73.0 400 succinyl-CoA synthetase beta chain 2823 6323 2727145 2726786 360  
2824 6324 2728133 2727399 735 gp:AF058302\_5 Streptomyces roseofulvus frnE 38.5  
71.8 213 frenolicin gene E product 2825 6325 2729025 2728207 819 2826 6326  
2730916 2729378 1539 sp:CAT1\_CLOKL Clostridium kluyveri cat1 cat1 47.9 77.8  
501 succinyl-CoA coenzyme A transferase 2827 6327 2731376 2732518 1143  
sp:NIR3\_AZOBZ Azospirillum brasilense ATCC 38.6 68.5 321 transcriptional  
regulator 29145 ntrC 2828 6328 2732230 2731424 807 2829 6329 2732636 2733367  
732 pir:E70810 Mycobacterium tuberculosis 46.5 81.7 213 phosphate transport  
system H37Rv Rv0821c phoY-2 regulatory protein 2830 6330 2734351 2733455 897  
pir:S68595 Pseudomonas aeruginosa pstB 58.8 82.8 255 phosphate-specific  
transport component 2831 6331 2735184 2734264 921 gp:MTPSTA1\_1 Mycobacterium  
tuberculosis 51.4 82.2 292 phosphate ABC transport system H37Rv Rv0830 pstA1  
permease protein 2832 6332 2736215 2735202 1014 pir:A70584 Mycobacterium  
tuberculosis 50.2 78.5 325 phosphate ABC transport system H37Rv Rv0829 pstC2  
permease protein 2833 6333 2737538 2736414 1125 pir:H70583 Mycobacterium  
tuberculosis 40.0 56.0 369 phosphate-binding protein S-3 H37Rv phoS2  
precursor 2834 6334 2738711 2737836 876 gp:SCD84\_18 Streptomyces coelicolor  
A3(2) 34.3 60.0 315 acetyltransferase SCD84.18c 2835 6335 2738771 2739553  
783 2836 6336 2740650 2739556 1095 sp:BMRU\_BACSU Bacillus subtilis 168 bmrU  
24.7 55.2 344 hypothetical protein 2837 6337 2740670 2741356 687 pir:E70809  
Mycobacterium tuberculosis 44.9 74.2 225 hypothetical protein H37Rv Rv0813c  
2838 6338 2742577 2741636 942 gp:AF193846\_1 Solanum tuberosum BCAT2 28.6 56.0  
259 branched-chain amino acid aminotransferase 2839 6339 2742685 2743785  
1101 gp:AB003158\_6 Corynebacterium 58.5 79.0 352 hypothetical protein  
ammoniogenes ATCC 6872 ORF4 2840 6340 2744010 2744222 213 pir:B70809  
Mycobacterium tuberculosis 58.6 81.0 58 hypothetical protein H37Rv Rv0810c  
2841 6341 2745954 2744881 1074 gp:AB003158\_5 Corynebacterium 81.0 94.2 347  
5'-phosphoribosyl-5-aminoimidazole ammoniogenes ATCC 6872 synthetase purM  
2842 6342 2747564 2746083 1482 gp:AB003158\_4 Corynebacterium 70.3 89.0 482

amidophosphoribosyl transferase ammoniagenes ATCC 6872 purF 2843 6343  
 2748057 2747683 375 pir:H70536 Mycobacterium tuberculosis 57.3 75.8 124  
 hypothetical protein H37Rv Rv0807 2844 6344 2748095 2749111 1017  
 gp:AB003158\_2 Corynebacterium 75.9 94.0 315 hypothetical protein ammoniagenes  
 ATCC 6872 ORF2 2845 6345 2749902 2749162 741 gp:AB003158\_1 Corynebacterium  
 67.7 87.1 217 hypothetical membrane protein ammoniagenes ATCC 6872 ORF1 2846  
 6346 2751918 2752103 186 GP:SSU18930\_21 **Sulfolobus** solfataricus 64.0 71.0 42  
 hypothetical protein 4 2847 6347 2752312 2750027 2286 gp:AB003162\_3  
 Corynebacterium 77.6 89.5 763 5'-phosphoribosyl-N- ammoniagenes ATCC 6872  
 formylglycinamide synthetase purL 2848 6348 2752402 2753121 720 2849 6349  
 2752995 2752327 669 gp:AB003162\_2 Corynebacterium 80.3 93.3 223  
 5'-phosphoribosyl-N- ammoniagenes ATCC 6872 formylglycinamide synthetase  
 purQ 2850 6350 2753237 2752995 243 gp:AB003162\_1 Corynebacterium 81.0 93.7 79  
 hypothetical protein ammoniagenes ATCC 6872 puror 2851 6351 2753298  
 2753819 522 2852 6352 2753804 2753328 477 prf:2420329A Lactococcus lactis gpo  
 46.2 77.9 158 glutathione peroxidase 2853 6353 2753992 2756739 2748  
 prf:2216389A Aeromonas hydrophila JMP636 28.0 51.5 965 extracellular nuclease  
 nucH 2854 6354 2756851 2757126 276 2855 6355 2757815 2757129 687 pir:C70709  
 Mycobacterium tuberculosis 37.4 68.7 211 hypothetical protein H37Rv Rv0784  
 2856 6356 2759200 2757863 1338 sp:DCTA\_SALTY Salmonella typhimurium LT2 49.0  
 81.6 414 C4-dicarboxylate transporter dctA 2857 6357 2761649 2759532 2118  
 prf:2408266A Pseudomonas sp. WO24 dapb1 41.8 70.6 697 dipeptidyl  
 aminopeptidase 2858 6358 2762452 2761829 624 2859 6359 2762675 2761785 891  
 gp:AB003161\_3 Corynebacterium 70.1 89.1 294 5'-phosphoribosyl-4-N-  
 ammoniagenes ATCC 6872 succinocarboxamide-5-amino purC imidazole synthetase  
 2860 6360 2764931 2763504 1428 gp:AB003161\_2 Corynebacterium 85.3 95.0 477  
 adenylosuccinoylase ammoniagenes ATCC 6872 purB 2861 6361 2766135 2764978  
 1158 sp:AAT\_SULSO **Sulfolobus** solfataricus ATCC 28.1 62.3 395 aspartate  
 aminotransferase 49255 2862 6362 2767420 2766158 1263 gp:AB003161\_1  
 Corynebacterium 71.1 86.4 425 5'-phosphoribosylglycinamide ammoniagenes ATCC  
 6872 synthetase purD 2863 6363 2767580 2767993 414 sp:YHIT\_MYCLE  
 Mycobacterium leprae u296a 53.7 80.2 136 histidine triad (HIT) family protein  
 2864 6364 2768137 2767703 435 2865 6365 2769095 2768343 753 pir:S62195  
 Methanosarcina barkeri orf3 26.8 56.4 243 hypothetical protein 2866 6366  
 2770511 2769156 1356 sp:DTPT\_LACLA Lactococcus lactis subsp. lactis 30.1 67.6  
 469 di-/tripeptide transporter dipT 2867 6367 2770714 2771982 1269  
 sp:BIOA\_CORGL Corynebacterium glutamicum 95.7 98.8 423  
 adenosylmethionine-8-amino-7- (Brevibacterium flavum) MJ233 oxononanoate  
 aminotransferase or bioA 7,8-diaminopelargonic acid aminotransferase 2868  
 6368 2771989 2772660 672 sp:BIOD\_CORGL Corynebacterium glutamicum 98.7 99.6  
 224 dethiobiotin synthetase (Brevibacterium flavum) MJ233 bioD 2869 6369  
 2774098 2772644 1455 gp:AF049873\_3 Lactococcus lactis M71plasmid 31.3 70.5 335  
 two-component system sensor pND306 histidine kinase 2870 6370

#### Detail Description Table CWU - DETL:

2774814 2774110 705 prf:2222216A Thermotoga maritima drrA 42.0 72.7 231  
 two-component system regulatory protein 2871 6371 2775689 2774937 753  
 sp:TIPA\_STRLI Streptomyces lividans tipA 37.4 69.5 249 transcriptional  
 activator 2872 6372 2776879 2775740 1140 prf:2419350A Arthrobacter sp. DK-38  
 30.9 53.9 382 metal-activated pyridoxal enzyme or low specificity D-Thr  
 aldolase 2873 6373 2778504 2776768 1737 gp:ECOPOXB8G\_1 Escherichia coli K12

poxB 46.3 75.8 574 pyruvate oxidase 2874 6374 2778965 2780446 1482  
 prf.2212334B Staphylococcus aureus plasmid 33.3 68.9 504 multidrug efflux  
 protein pSK23 qacB 2875 6375 2780439 2780969 531 sp:YCDC\_ECOLI Escherichia  
 coli K12 ycdC 30.4 68.5 92 transcriptional regulator 2876 6376 2780996  
 2782315 1320 pir:D70551 Mycobacterium tuberculosis 45.6 78.4 421 hypothetical  
 membrane protein H37Rv Rv2508c 2877 6377 2784481 2782340 2142 2878 6378  
 2785615 2784656 960 gp.AF096929\_2 Rhodococcus erythropolis SQ1 34.3 62.1 303  
 3-ketosteroid dehydrogenase kstD1 2879 6379 2786355 2785651 705  
 sp.ALSR\_BACSU Bacillus subtilis 168 alsR 37.1 69.0 232 transcriptional  
 regulator, LysR family 2880 6380 2787782 2788594 813 pir:C70982 Mycobacterium  
 tuberculosis 28.4 52.9 278 hypothetical protein H37Rv Rv3298c lpqC 2881 6381  
 2789399 2788587 813 pir:C69862 Bacillus subtilis 168 ykrA 26.7 55.6 288  
 hypothetical protein 2882 6382 2789935 2789477 459 2883 6383 2790152 2790550  
 399 pir:A45264 Oryctolagus cuniculus kidney 28.6 50.7 140 hypothetical protein  
 cortex rBAT 2884 6384 2790946 2792448 1503 pir:B70798 Mycobacterium  
 tuberculosis 36.0 64.0 464 hypothetical membrane protein H37Rv Rv3737 2885  
 6385 2792531 2792857 327 pir:S41307 Streptomyces griseus hrdB 32.3 50.3 155  
 transcription initiation factor sigma 2886 6386 2792873 2794327 1455  
 sp:TPS1\_SCHPO Schizosaccharomyces pombe 38.8 66.7 487 **trehalose**-6-phosphate  
 synthase tps1 2887 6387 2794300 2794812 513 2888 6388 2794870 2795637 768  
 sp:OTSB\_ECOLI Escherichia coli K12 otsB 27.4 57.6 245 **trehalose**-phosphatase  
 2889 6389 2796749 2795676 1074 sp:CCPA\_BACME Bacillus megaterium ccpA 24.7  
 60.2 344 glucose-resistance amylase regulator 2890 6390 2796865 2797806 942  
 sp:ZNUA\_HAEIN Haemophilus influenzae Rd 22.4 46.7 353 high-affinity zinc  
 uptake system HI0119 znuA protein 2891 6391 2797820 2798509 690  
 gp:AF121672\_2 Staphylococcus aureus 8325-4 31.4 63.2 223 ABC transporter mreA  
 2892 6392 2798837 2799391 555 pir:E70507 Mycobacterium tuberculosis 60.0 87.4  
 135 hypothetical membrane protein H37Rv Rv2060 2893 6393 2799535 2801034 1500  
 pir:A69426 Archaeoglobus fulgidus 23.4 52.5 303 transposase (ISA0963-5) 2894  
 6394 2801113 2801313 201 2895 6395 2803246 2801558 1689 gp:AF096929\_2  
 Rhodococcus erythropolis SQ1 32.1 62.0 561 3-ketosteroid dehydrogenase kstD1  
 2896 6396 2803996 2803250 747 2897 6397 2804691 2804074 618 pir:B72359  
 Thermotoga maritima MSB8 34.3 56.4 204 lipopolysaccharide biosynthesis bplA  
 protein or oxidoreductase or dehydrogenase 2898 6398 2805110 2804676 435  
 sp:MI2D\_BACSU Bacillus subtilis 168 idh or iolG 35.2 69.5 128 dehydrogenase or  
 myo-inositol 2- dehydrogenase 2899 6399 2805967 2805113 855 sp:SHIA\_ECOLI  
 Escherichia coli K12 shiA 30.5 67.5 292 shikimate transport protein 2900 6400  
 2806441 2806016 426 sp:SHIA\_ECOLI Escherichia coli K12 shiA 43.1 80.8 130  
 shikimate transport protein 2901 6401 2807252 2806599 654 gp:SC5A7\_19  
 Streptomyces coelicolor A3(2) 32.6 55.7 212 transcriptional regulator  
 SC5A7.19c 2902 6402 2808364 2807426 939 sp:PT56\_YEAST Saccharomyces  
 cerevisiae 22.8 47.3 334 ribosomal RNA ribose methylase or YOR201C PET56  
 tRNA/rRNA methyltransferase 2903 6403 2809778 2808399 1380 sp:SYC\_ECOLI  
 Escherichia coli K12 cysS 42.2 68.8 464 cysteinyl-tRNA synthetase 2904 6404  
 2811806 2809824 1983 prf:2511335C Lactococcus lactis sacB 47.0 77.0 668 PTS  
 system, enzyme II sucrose protein (sucrose-specific IIABC component) 2905  
 6405 2813258 2811960 1299 gp:AF205034\_4 Clostridium acetobutylicum 35.3 56.9  
 473 sucrose 6-phosphate hydrolase or ATCC 824 scrB sucrose 2906 6406 2814037  
 2813279 759 sp:NAGB\_ECOLI Escherichia coli K12 nagB 38.3 69.4 248  
 glucosamine-6-phosphate isomerase 2907 6407 2815232 2814081 1152  
 sp:NAGA\_VIBFU Vibrio furnissii SR1514 manD 30.2 60.3 368  
 N-acetylglucosamine-6-phosphate deacetylase 2908 6408 2815458 2816393 936  
 sp:DAPA\_ECOLI Escherichia coli K12 dapA 28.2 62.1 298 dihydrodipicolinate

synthase 2909 6409 2816409 2817317 909 sp:GLK\_STRCO *Streptomyces coelicolor*  
 A3(2) 28.7 57.6 321 glucokinase SC6E10.20c glk 2910 6410 2817363 2818058  
 696 prf:2516292A *Clostridium perfringens* NCTC 36.4 68.6 220  
 N-acetylmannosamine-6-phosphate 8798 nanE epimerase 2911 6411 2818313 2818137  
 177 2912 6412 2819564 2818350 1215 sp:NANH\_MICVI *Micromonospora viridifaciens*  
 24.8 50.3 439 sialidase precursor ATCC 31146 nadA 2913 6413 2820285 2819557  
 729 gp:AF181498\_1 *Rhizobium etli* ansR 26.6 57.2 222 L-asparagine permease  
 operon repressor 2914 6414 2820584 2822191 1608 gp:BFU64514\_1 *Bacillus*  
*firmus* OF4 dppA 22.5 51.4 560 dipeptide transporter protein or heme-binding  
 protein 2915 6415 2822387 2823337 951 sp:DPPB\_BACFI *Bacillus firmus* OF4 dappB  
 31.9 64.3 342 dipeptide transport system permease protein 2916 6416 2824274  
 2825341 1068 sp:OPPD\_BACSU *Bacillus subtilis* 168 oppD 46.5 78.3 314  
 oligopeptide transport ATP-binding protein 2917 6417 2825341 2826156 816  
 sp:OPPF\_LACLA *Lactococcus lactis* oppF 43.4 78.7 258 oligopeptide transport  
 ATP-binding protein 2918 6418 2826835 2826215 621 sp:RHTB\_ECOLI *Escherichia*  
*coli* K12 rhtB 28.5 62.7 193 homoserine/homoserin lactone efflux protein or  
 lysE type translocator 2919 6419 2826922 2827404 483 prf:2309303A  
*Bradyrhizobium japonicum* lrp 31.0 66.2 142 leucine-responsive regulatory  
 protein 2920 6420 2827817 2827458 360 2921 6421 2828383 2827904 480  
 pir:C70607 *Mycobacterium tuberculosis* 55.9 86.2 152 hypothetical protein H37Rv  
 Rv3581c 2922 6422 2829146 2828379 768 sp:Y18T\_MYCTU *Mycobacterium*  
*tuberculosis* 46.4 71.5 235 hypothetical protein H37Rv Rv3582c 2923 6423  
 2829749 2829156 594 pir:H70803 *Mycobacterium tuberculosis* 73.3 91.1 157  
 transcription factor H37Rv Rv3583c 2924 6424 2830057 2830779 723  
 prf:2214304A *Mycobacterium tuberculosis* 43.5 70.0 223 two-component system  
 response H37Rv Rv3246c mtrA regulator 2925 6425 2830779 2831894 1116  
 sp:BAES\_ECOLI *Escherichia coli* K12 baeS 29.3 67.7 341 two-component system  
 sensor histidine kinase 2926 6426 2832085 2832666 582 2927 6427 2832790  
 2834181 1392 sp:RADA\_ECOLI *Escherichia coli* K12 radA 41.5 74.3 463 DNA repair  
 protein RadA 2928 6428 2834188 2835285 1098 sp:YACK\_BACSU *Bacillus subtilis*  
 168 yackK 40.3 73.3 345 hypothetical protein 2929 6429 2835969 2835283 687  
 pir:D70804 *Mycobacterium tuberculosis* 29.4 53.3 231 hypothetical protein  
 H37Rv Rv3587c 2930 6430 2837499 2836048 1452 gp:PPU96338\_1 *Pseudomonas putida*  
 NCIMB 59.5 85.1 471 p-hydroxybenzaldehyde 9866 plasmid pRA4000 dehydrogenase  
 2931 6431 2837737 2837591 147 2932 6432 2838576 2837956 621 pir:T08204  
*Chlamydomonas reinhardtii* ca1 36.7 66.2 210 mitochondrial carbonate  
 dehydratase beta 2933 6433 2838643 2839521 879 gp:AF121797\_1 *Streptomyces*  
*antibioticus* IMRU 48.4 70.7 283 A/G-specific adenine glycosylase 3720 mutY  
 2934 6434 2839562 2840716 1155 2935 6435 2841063 2840758 306 2936 6436  
 2841075 2841848 774 gp:AB009078\_1 *Brevibacterium saccharolyticum* 99.2 99.6 258  
 L-2,3-butanediol dehydrogenase 2937 6437 2842130 2842453 324 2938 6438  
 2842493 2843233 741 2939 6439 2843405 2843716 312 2940 6440 2843722 2843432  
 291 pir:E70552 *Mycobacterium tuberculosis* 48.5 69.1 97 hypothetical protein  
 H37Rv Rv3592 2941 6441 2845139 2845558 420 GSP:Y29188 *Pseudomonas aeruginosa*  
 57.0 63.0 99 virulence factor ORF24222 2942 6442 2845889 2846101 213  
 GSP:Y29193 *Pseudomonas aeruginosa* 54.0 55.0 72 virulence factor ORF25110  
 2943 6443 2846186 2846506 321 GSP:Y29193 *Pseudomonas aeruginosa* 74.0 75.0 55  
 virulence factor ORF25110 2944 6444 2846940 2844166 2775 sp:MECB\_BACSU  
*Bacillus subtilis* 168 mecB 58.5 86.2 832 ClpC adenosine triphosphatase/  
 ATP-binding proteinase 2945 6445 2847229 2848659 1431 gp:AB035643\_1 *Bacillus*  
*cereus* ts-4 impdh 37.1 70.2 469 inosine monophosphate dehydrogenase 2946  
 6446 2848769 2849779 1011 pir:JC6117 *Rhodococcus rhodochrous* nitR 24.7 62.7  
 316 transcription factor 2947 6447 2850031 2851815 1785 sp:PH2M\_TRICU

Trichosporon cutaneum ATCC 33.5 60.9 680 phenol 2-monooxygenase 46490 2948  
6448 2852017 2853732 1716 2949 6449 2853769 2855709 1941 2950 6450 2855795  
2857516 1722 2951 6451 2859044 2859205 162 2952 6452 2859055 2857613 1443  
gp:AF237667\_1 Corynebacterium glutamicum 100.0 100.0 481 lincomycin resistance  
protein ImrB 2953 6453 2860145 2859195 951 pir:G70807 Mycobacterium  
tuberculosis 26.7 55.8 240 hypothetical protein H37Rv Rv3517 2954 6454  
2862082 2860505 1578 gp:AB012100\_1 Bacillus stearothermophilus lysS 41.7 71.2  
511 lysyl-tRNA synthetase 2955 6455 2862929 2862132 798 gp:CGPAN\_2  
Corynebacterium glutamicum 29.9 52.6 268 pantoate-beta-alanine ligase

PGPUB-DOCUMENT-NUMBER: 20020164723

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164723 A1

TITLE: Method of producing saccharide preparations

PUBLICATION-DATE: November 7, 2002

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APPL-NO: 09/ 908395

DATE FILED: July 18, 2001

RELATED-US-APPL-DATA:

child 09908395 A1 20010718 parent continuation-of 09632392 20000804 US GRANTED  
parent-patent 6303346 US child 09632392 20000804 US parent continuation-of  
09499531 20000210 US GRANTED parent-patent 6136571 US child 09499531 20000210  
US parent continuation-of 09198672 19981123 US GRANTED parent-patent 6129788 US  
child 09198672 19981123 US parent continuation-in-part-of 09107657 19980630 US  
ABANDONED child 09107657 19980630 US parent continuation-in-part-of 08979673  
19971126 US ABANDONED

US-CL-CURRENT: 435/96

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.



## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/632,392, filed on Aug. 4, 2000, now allowed, which is a continuation of U.S. patent application Ser. No. 09/499,531, filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. patent application Ser. No. 09/198,672, filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. patent application Ser. No. 09/107,657, filed on Jun. 30, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/979,673, filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference.

[0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

[0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

----- KWIC -----

### Cross Reference to Related Applications Paragraph - CRTX:

[0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

### Cross Reference to Related Applications Paragraph - CRTX:

[0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent

steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

Summary of Invention Paragraph - BSTX:

[0022] Trehalose syrups

Summary of Invention Paragraph - BSTX:

[0023] Trehalose (.alpha.-D-glucopyranosyl .alpha.-D-glucopyranoside) is a non reducing disaccharide with two glucose residues bound by a .alpha.-1,1 linkage.

Summary of Invention Paragraph - BSTX:

[0024] Enzymatic processes for producing trehalose from starch or maltooligosaccharides are described by, e.g., Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550; Kazuhisa et al. (1997), Starch 49, no. 1. p. 26-30; and in EP 764,720.

Summary of Invention Paragraph - BSTX:

[0039] It has now been found that in a method of producing mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, the efficiency can be improved significantly, and the costs lowered, if in the saccharification (or hydrolyzing) step, after the liquefaction step, the syrup is subjected to one or more high temperature membrane separation steps, and the saccharification enzyme is returned to the saccharification step. According to the method of the present invention, the membrane separation step may be regarded as an integral part of the saccharification step.

Summary of Invention Paragraph - BSTX:

[0042] When producing saccharides with more than one saccharide unit, i.e., trehalose, isomaltooligosaccharides, cyclodextrins and maltooligosaccharides the hydrolyzing step (after the liquefaction step) is followed by an ultra and microfiltration step or a micro and ultrafiltration step.

Summary of Invention Paragraph - BSTX:

[0047] In its second aspect, the invention provides a method for the production of a mono and/or oligosaccharide preparation of, e.g., dextrose, trehalose, isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, which method comprises an enzymatic saccharification step, and the subsequent steps of

Detail Description Paragraph - DETX:

[0068] According to the method of the invention, the retentate from the membrane separation is conveyed back (re-circulated) to the saccharification step. Preferably the retentate from the membrane separation is re-circulated to a saccharification stage in the saccharification step, at which stage the content of the reaction mixture matches the content of the retentate with respect to the saccharide, such as glucose, trehalose, isomaltooligosaccharide, cyclodextrin or maltooligosaccharide.

Detail Description Paragraph - DETX:

[0073] When producing trehalose, the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% trehalose.

Detail Description Paragraph - DETX:

[0076] In the context of the present invention a membrane separation step comprises a microfiltration step followed by an ultrafiltration step or an ultrafiltration step followed by a microfiltration step when producing trehalose, isomaltooligosaccharides, cyclodextrins and malto-oligosaccharides.

Detail Description Paragraph - DETX:

[0079] In a preferred embodiment, the membrane separation steps comprises a microfiltration step and an ultrafiltration step, applied in the order specified. This embodiment is particularly useful for the production of a syrup holding from about 95 to about 96% glucose, or from 10-40% isomaltose, or 30 to above 80% maltose, or 75-90% trehalose, or 30-60% cyclodextrins.

Detail Description Paragraph - DETX:

[0102] Production of trehalose (containing 75-90% trehalose)

Detail Description Paragraph - DETX:

[0103] In the saccharification step, when producing trehalose, liquefied starch is subjected to the action of an enzyme capable of first converting maltooligosaccharide (from the liquefaction step) into the non reducing saccharide maltooligosyl trehalose by intramolecular transglycosylation followed by a subsequent step of hydrolyzing the reaction product of the first step (i.e., maltooligosyl trehalose) into trehalose. The saccharification step may be performed using maltooligosyl trehalose synthase (MTSase) and maltooligosyl trehalose trehalohydrolase (MTHase), e.g., the two enzymes described by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550). MTSase and MTHase act on amylose or starch to produce trehalose.

Detail Description Paragraph - DETX:

[0104] Another enzymatic process for producing trehalose from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550) involves using trehalose-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae Sulfolobus solfataricus KM1.

Detail Description Paragraph - DETX:

[0105] Further, EP 764720 also describes using two enzymes from Sulfolobus spp. for preparing trehalose from starch or maltooligosaccharides.

Detail Description Paragraph - DETX:

[0124] When producing trehalose, the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% trehalose.

Detail Description Paragraph - DETX:

[0141] A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic archaeobacterium Sulfolobus acidocaldarius (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as Sulfolobus acidocaldarius ATCC33909 and from a strain of Rhodothermus maris.

Detail Description Paragraph - DETX:

[0153] Preferably, the saccharification step, when producing trehalose is performed in presence of a MTSase and MTHase, e.g., the enzymes disclosed by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550).

Claims Text - CLTX:

33. The method according to claim 32, in which the MTSase and MTHase is derived from a strain of Sulfolobus, such as S. Acidocaldarius, especially S. acidocaldarius ATCC 33909.

Claims Text - CLTX:

44. The method according to claim 31, for the production of a trehalose preparation holding of from about 75 to about 90% trehalose.

Claims Text - CLTX:

79. The method according to claim 78, in which the MTSase and MTHase is derived from a strain of Sulfolobus, such as S. acidocaldarius, especially S. acidocaldarius ATCC 33909.

Claims Text - CLTX:

90. The method according to claim 77, for the production of a trehalose preparation holding of from about 75 to about 90% trehalose.

PGPUB-DOCUMENT-NUMBER: 20020160402

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160402 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen	Dallas	TX	US	
Albert				

APPL-NO: 10/ 077392

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077392 A1 20020215 parent division-of 09535366 20000324 US PENDING  
non-provisional-of-provisional 60125864 19990324 US  
non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/6,435/320.1 ,435/325 ,435/455 ,435/69.1 ,435/7.1

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0092] In certain embodiments, the organism is an archaea (a.k.a.

archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus**, a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0203] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. Azuma et al., (1988) show that **trehalose** dimycolate administration correlates with augmented resistance to influenza virus infection in mice. **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0208] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with **trehalose** dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0221] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, gamma-interferon, GMCSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020155508

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155508 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen Albert	Dallas	TX	US	

APPL-NO: 10/ 077247

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077247 A1 20020215 parent division-of 09535366 20000324 US GRANTED  
parent-patent 6410241 US non-provisional-of-provisional 60125864 19990324 US  
non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/7.2,435/455 ,435/6 ,435/91.2

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0095] In certain embodiments, the organism is an archaea (a.k.a.



archaebacteria; e.g., a methanogens, a halophiles, a sulfolobus). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a sulfolobus, a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

#### Detail Description Paragraph - DETX:

[0206] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without trehalose dimycolate. Trehalose dimycolate may be used itself. Azuma et al., (1988) show that trehalose dimycolate administration correlates with augmented resistance to influenza virus infection in mice. Trehalose dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

#### Detail Description Paragraph - DETX:

[0211] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with trehalose dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with trehalose dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and trehalose dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and trehalose dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

#### Detail Description Paragraph - DETX:

[0224] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020151060

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020151060 A1

TITLE: PEI: DNA vector formulations for in vitro and in vivo gene delivery

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cristiano, Richard J.	Pearland	TX	US	
Yamashita, Motoyuki	Kochi City		JP	

APPL-NO: 09/ 962922

DATE FILED: September 25, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60235237 20000925 US  
non-provisional-of-provisional 60235635 20000926 US

US-CL-CURRENT: 435/455,424/486 ,514/44

ABSTRACT:

The present invention relates generally to the fields of nucleic acid transfection. More particularly, it concerns novel polycation:nucleic acid compositions, methods of preparation of such compositions and methods of transfecting cells with such compositions.

[0001] This application claims the priority of U.S. Provisional Patent Application Ser. No. 60/235,237, filed Sep. 25, 2000 and U.S. Provisional Patent Application Ser. No. 60/235,635, filed Sep. 26, 2000, both of which disclosures are specifically incorporated herein by reference in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0275] In certain embodiments, the organism is an archaea (a.k.a. archaeobacteria; e.g., a methanogens, a halophiles, a sulfolobus). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a sulfolobus, a metallosphaera, an acidianus, a

thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0482] Another adjuvant contemplated for use in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without trehalose dimycolate. Trehalose dimycolate may be used itself. Trehalose dimycolate administration has been shown to correlate with augmented resistance to influenza virus infection in mice (Azuma et al., 1988). Trehalose dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0487] One group of adjuvants preferred for use in the invention are the detoxified endotoxins, such as the refined detoxified endotoxin of U.S. Pat. No. 4,866,034. These refined detoxified endotoxins are effective in producing adjuvant responses in mammals. Of course, the detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. For example, combination of detoxified endotoxins with trehalose dimycolate is particularly contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with trehalose dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and trehalose dimycolate; as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and trehalose dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

PGPUB-DOCUMENT-NUMBER: 20020150940

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020150940 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen	Dallas	TX	US	
Albert				

APPL-NO: 10/ 077232

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077232 A1 20020215 parent continuation-of 09535366 20000324 US GRANTED  
parent-patent 6410241 US non-provisional-of-provisional 60125864 19990324 US  
non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/6,435/455 ,435/7.1 ,435/91.2

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0092] In certain embodiments, the organism is an archaea (a.k.a.

archaeobacteria; e.g., a methanogens, a halophiles, a sulfolobus). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a sulfolobus, a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0203] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without trehalose dimycolate. Trehalose dimycolate may be used itself. Azuma et al., (1988) show that trehalose dimycolate administration correlates with augmented resistance to influenza virus infection in mice. Trehalose dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0208] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with trehalose dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with trehalose dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and trehalose dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and trehalose dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0221] Adjuvants that may be used include IL-1, IL-2, IL4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020146733

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146733 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen	Dallas	TX	US	
Albert				

APPL-NO: 10/ 077621

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077621 A1 20020215 parent division-of 09535366 20000324 US PENDING  
non-provisional-of-provisional 60125864 19990324 US  
non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/6,435/455 ,435/69.1 ,435/7.1 ,435/91.2

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

----- KWIC -----

Detail Description Paragraph - DETX:

[0092] In certain embodiments, the organism is an archaea (a.k.a.

archaebacteria; e.g., a methanogens, a halophiles, a sulfolobus). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a sulfolobus, a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0203] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without trehalose dimycolate. Trehalose dimycolate may be used itself. Azuma et al., (1988) show that trehalose dimycolate administration correlates with augmented resistance to influenza virus infection in mice. Trehalose dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0208] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with trehalose dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with trehalose dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and trehalose dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and trehalose dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0221] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, gamma-interferon, GM-CSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020120116

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020120116 A1

TITLE: ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
KUNSCH, CHARLES A.	ATLANTA	GA	US	
DILLON, PATRICK J.	CARLSBAD	CA	US	
BARASH, STEVEN	ROCKVILLE	MD	US	

APPL-NO: 09/ 070927

DATE FILED: May 4, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 536/23.2, 435/252.3, 435/320.1, 435/69.1, 435/70.1, 435/71.1, 530/350, 530/387.9, 800/13

ABSTRACT:

The present invention provides polynucleotide sequences of the genome of *Enterococcus faecalis*, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

[0001] This application claims benefit of 35 U.S.C. section 119(e) based on copending U.S. Provisional Application Serial No. 60/046,655, filed May 16, 1997; 60/044,031, filed May 6, 1997; and 60/066,099, filed Nov. 14, 1997. Provisional Application Serial No. 60/066,099, filed Nov. 14, 1997 is herein incorporated by reference in its entirety.

----- KWIC -----

Detail Description Table CWU - DETL:

product [*Bacillus subtilis*] 68 53 363 8 12517 9950 gi.vertline.1652980  
H(+)-transporting ATPase [*Synechocystis* 68 46 sp.] 368 3 1269 1736



gnl.vertline.PID.vertline.e209005 homologous to ORF2 in nrdEF operons of 68 37  
 E.coli and S.typhimurium [Lactococcus lactis] 386 11 6564 6115  
 gi.vertline.765072 ORF3 [Staphylococcus aureus] 68 46 395 3 935 729  
 gi.vertline.5521 ORF 3 (AA 1-90) [Bacteriophage phi-105] 68 34 399 8 6073 6519  
 gi.vertline.153584 biotin carboxyl carrier protein 68 53 [Streptococcus  
 mutans] sp.vertline.P29337.vertlin- e.BCCP\_STRMU BIOTIN CARBOXYL CARRIER  
 PROTEIN (BCCP). 408 3 2289 1336 gi.vertline.41572 GlnP (AA 1-219) [Escherichia  
 coli] 68 40 420 1 559 2 gi.vertline.1592142 ABC transporter, probable  
 ATP-binding 68 51 subunit [Methanococcus jannaschii] 423 2 254 1294  
 gi.vertline.1773109 similar to S. typhimurium apbA 68 47 [Escherichia coli]  
 423 3 1465 2421 gi.vertline.1653032 hypothetical protein [Synechocystis sp.]  
 68 40 428 1 859 2 gi.vertline.1652454 hypothetical protein [Synechocystis  
 sp.] 68 48 432 7 4626 3901 gi.vertline.1573285 hypothetical [Haemophilus  
 influenzae] 68 55 434 1 90 1889 gi.vertline.1542975 AbcB  
 [Thermoanaerobacterium 68 50 thermosulfurigenes] 441 5 4674 5156  
 gi.vertline.467437 unknown [Bacillus subtilis] 68 48 455 4 3835 4080  
 gi.vertline.19815 luminal binding protein (BiP) [Nicotiana 68 40 tabacum]  
 530 2 394 546 gi.vertline.763326 unknown [Saccharomyces cerevisiae] 68 42  
 531 2 810 622 gi.vertline.1146183 putative [Bacillus subtilis] 68 51 537 3  
 1353 1192 gi.vertline.929968 ORFA; similar to B. anthracis WeyAR 68 56  
 element ORFA; putative transposase [Bacillus anthracis] 539 3 2725 2231  
 gi.vertline.1353537 dUTPase [Bacteriophage rit] 68 53 569 1 3 446  
 gi.vertline.146544 18 kD protein [Escherichia coli] 68 47 591 2 656 174  
 gi.vertline.1039479 ORFU [Lactococcus lactis] 68 42 652 2 739 1032  
 gi.vertline.1303715 YrkP [Bacillus subtilis] 68 50 671 2 436 1617  
 gi.vertline.413959 ipa-35d galK gene product [Bacillus 68 50 subtilis] 684 1  
 466 2 gnl.vertline.PID.vertline.e248400 orfRM1 gene product [Bacillus  
 subtilis] 68 40 693 1 2 787 gi.vertline.405804 transposase [Streptococcus  
 thermophilus] 68 46 700 2 772 596 gi.vertline.153801 enzyme scr-II  
 [Streptococcus mutans] 68 50 735 1 118 609 gi.vertline.969027  
 gamma-aminobutyrate permease [Bacillus 68 40 subtilis]  
 sp.vertline.P46349.vertline.GABP\_BACSU GABA PERMEASE (4-AMINO BUTYRATE  
 TRANSPORT CARRIER) (GABA-AMINO BUTYRATE PERMEASE). 750 1 2 529  
 gi.vertline.893358 PgsA [Bacillus subtilis] 68 54 762 2 1588 950  
 gi.vertline.1146240 ketopantoate hydroxymethyltransferase 68 49 [Bacillus  
 subtilis] 790 1 407 3 gi.vertline.142224 attachment protein ChvA (ttg start  
 codon) 68 55 [Agrobacterium tumefaciens] 882 1 3 278 gi.vertline.57572  
 glyceraldehyde-3-phosphate dehydrogenase 68 48 (NADP+) (phosphorylating)  
 attus rattus] 950 1 140 568 gi.vertline.882736 ORF\_f278 [Escherichia coli] 68  
 53 969 2 554 339 gi.vertline.1118031 similar to neural cell adhesion  
 molecules 68 47 and neuroglycans in their IG-like C2-type domains  
 [Caenorhabditis elegans] 970 1 297 73 gi.vertline.474404 cyclophilin  
 [Tolypocladium inflatum] 68 40 1 1 1103 3 gi.vertline.48790 ORF 3  
 [Pseudomonas putida] 67 50 29 10 7156 6614  
 sp.vertline.P36672.vertline.PTTB\_ECO PTS SYSTEM, TREHALOSE-SPECIFIC IIBC 67 52  
 LI COMPONENT (EIIBC-TRE) (TREHALOSE- PERMEASE IIBC COMPONENT)  
 (PHOSPHOTRANSFERASE ENZYME II, BC COMPONENT) (EC 2.7.1.69) (EII-TRE). 48 8  
 8035 9141 gi.vertline.975627 N-acylamino acid racemase [Amycolatopsis 67 48  
 sp.] 55 12 6621 7439 gi.vertline.391610 farnesyl diphosphate synthase  
 [Bacillus 67 47 stearothermophilus] pir.vertline.JX0257.vertline.JX0257  
 geranyltranstransferase (BC 2.5.1.10) - Bacillus stearothermophilus 57 13  
 13972 16401 gnl.vertline.PID.vertline.e255- 138 phenylalanyl-tRNA synthetase  
 beta subunit 67 47 [Bacillus subtilis] 63 4 1917 2729 gi.vertline.1321629

MIP related protein of *E. coli* 67 47 [*Escherichia coli*] 68 12 8600 8923  
gi.vertline.793910 surface antigen [*Homo sapiens*] 67 43 72 7 7138 6740  
gnl.vertline.PID.vertline.e209005 homologous to ORF2 in *nrdEF* operons of 67 39  
*E. coli* and *S. typhimurium* [*Lactococcus lactis*] 72 10 8309 9433  
gi.vertline.1199515 ferrous iron transport protein B 67 41 [*Escherichia coli*]  
85 5 5315 4296 gi.vertline.142611 branched chain alpha-keto acid 67 52  
dehydrogenase E1-alpha [*Bacillus subtilis*] 101 5 4149 3100 gi.vertline.1109686  
ProX [*Bacillus subtilis*] 67 48 110 4 2335 1292 gi.vertline.1066343  
mu-crystallin [*Homo sapiens*] 67 48 114 12 12936 13520 gi.vertline.146218  
serine hydroxymethyltransferase 67 50 [*Escherichia coli*] 115 5 3137 2010  
gi.vertline.1256150 YbaR [*Bacillus subtilis*] 67 47 115 6 3199 2792  
gi.vertline.1652593 hypothetical protein [*Synechocystis* sp.] 67 45 123 25  
22739 24208 gi.vertline.148711 6-aminohexanoate-cyclic-dimer hydrolase 67 50  
[*Flavobacterium* sp.] gi.vertline.488343 6-aminohexanoate-cyclic-dimer  
hydrolase [*Flavobacterium* p.] 124 6 5139 4267 gi.vertline.1016770  
prolipoprotein diacylglycerol transferase 67 50 [*Staphylococcus aureus*] 125  
2 1306 221 gi.vertline.853743 L-alanoyl-D-glutamate peptidase 67 50  
[Bacteriophage A118] 128 36 29462 28737 gi.vertline.142940 ftsA [*Bacillus*  
subtilis] 67 46 138 27 17602 18183 gi.vertline.1256639 putative [*Bacillus*  
subtilis] 67 50 138 31 21578 20097 gi.vertline.143245 Na<sup>+</sup>/H<sup>+</sup> antiporter  
[*Bacillus firmus*] 67 42 138 33 25165 23249 gi.vertline.1498811 M. jannaschii  
predicted coding region 67 45 MJ0050 [*Methanococcus jannaschii*] 138 36 28690  
27362 gnl.vertline.PID.vertline.e269549 Unknown [*Bacillus subtilis*] 67 47 144  
4 3271 3717 gi.vertline.1753229 PKCI [*Borrelia burgdorferi*] 67 52 145 3 1435  
2511 gi.vertline.1573615 ATP-binding protein (abc) [*Haemophilus* 67 47  
influenzae] 146 5 4657 2804 gi.vertline.1045034 beta-galactosidase  
[*Xanthomonas campestris* 67 51 pv. manihotis] 149 3 1978.1367  
gi.vertline.806536 membrane protein [*Bacillus* 67 51 acidopullulyticus] 156 1  
3 365 gnl.vertline.PID.vertline.e265539 ClpB-homologue [*Thermus aquaticus* 67  
42 thermophilus] 158 15 14863 13766 gi.vertline.1573487 rbs repressor (rbsR)  
[*Haemophilus* 67 40 influenzae] 158 17 16483 15959 gi.vertline.677850  
hypothetical protein [*Staphylococcus* 67 51 aureus] 159 7 6872 6006  
gi.vertline.1303949 YqiX [*Bacillus subtilis*] 67 41 159 9 8103 7498  
gi.vertline.1303950 YqiY [*Bacillus subtilis*] 67 41 165 11 9846 9004  
gi.vertline.606079 ORF\_o267 [*Escherichia coli*] 67 36 169 2 2151 3047  
gi.vertline.42371 pyruvate formate-lyase activating enzyme 67 44 (AA 1-246)  
[*Escherichia coli*] 179 13 13648 14451 gnl.vertline.PID.vertline.e257631  
methyltransferase [*Lactococcus lactis*] 67 45 180 28 28656 29801  
gi.vertline.666005 hypothetical protein [*Bacillus subtilis*] 67 48 194 6 2774  
4231 gi.vertline.143245 Na<sup>+</sup>/H<sup>+</sup> antiporter [*Bacillus firmus*] 67 41 194 10 6472  
8259 gi.vertline.622991 mannitol transport protein [*Bacillus* 67 50  
stearothermophilus] sp.vertline.P50852.vertline.PTMB\_BACST PTS SYSTEM,  
MANNITOL-SPECIFIC IIBC COMPONENT EIIBC-MTL) (MANNITOL-PERMEASE IIBC  
COMPONENT) (PHOSPHOTRANSFERASE NZYME II, BC COMPONENT) (EC 2.7.1.69)  
(EII-MTL). 204 5 1924 3006 gi.vertline.1235684 mevalonate pyrophosphate  
decarboxylase 67 50 [*Saccharomyces cerevisiae*] 214 1 42 1196  
gi.vertline.606013 CG Site No. 829 [*Escherichia coli*] 67 36 219 2 524 850  
gnl.vertline.PID.vertline.e257628 ORF [*Lactococcus lactis*] 67 42 223 15 13640  
14407 gi.vertline.496520 orf iota [*Streptococcus pyogenes*] 67 54 227 3 1011  
1892 gi.vertline.1070013 protein-dependent [*Bacillus subtilis*] 67 37 233 12  
9340 8339 gi.vertline.507880 xanthine dehydrogenase [*Gallus gallus*] 67 50 238  
10 7951 9183 gi.vertline.1653948 hypothetical protein [*Synechocystis* sp.] 67  
45 246 3 783 1430 gnl.vertline.PID.vertline.e233869 hypothetical protein

[*Bacillus subtilis*] 67 47 256 2 570 1601 gi.vertline.709992 hypothetical protein [*Bacillus subtilis*] 67 36 266 2 1266 835 gi.vertline.963038 ArpU [*Enterococcus hirae*] 67 42 285 1 3 809 gi.vertline.40014 pot. ORF 446 (aa 1-446) [*Bacillus subtilis*] 288 10 6838 5801 gi.vertline.1651806 hypothetical protein [*Synechocystis* sp.] 67 45 301 10 8822 8562 gi.vertline.1303864 YqqQ [*Bacillus subtilis*] 67 43 312 5 2377 2595 gi.vertline.709991 hypothetical protein [*Bacillus subtilis*] 67 52 353 1 3 1472 gi.vertline.151259 HMG-CoA reductase (EC 1.1.1.88) 67 48 [*Pseudomonas mevalonii*] pir.vertline.A44756.vertline.A44756 hydroxymethylglutaryl-CoA reductase (EC 1.1.1.88) *Pseudomonas* sp. 359 2 984 439 gi.vertline.1773190 similar to *E. coli* yhaE [*Escherichia coli*] 67 45 359 3 2244 982 gi.vertline.1001478 hypothetical protein [*Synechocystis* sp.] 67 30 364 8 8469 7816 gi.vertline.496943 ORF [*Saccharomyces cerevisiae*] 67 50 386 12 6625 7833 gnl.vertline.PID.vertline.e254644 membrane protein [*Streptococcus pneumoniae*] 394 2 497 2635 gnl.vertline.PID.vertline.e25593 hypothetical protein [*Bacillus subtilis*] 67 45 399 6 5410 3971 gi.vertline.665994 hypothetical protein

#### Detail Description Table CWU - DETL:

pir.vertline.S20433.vertline.S20433 Isp protein - *Staphylococcus aureus* sp.vertline.P31024.vertline.LSPA\_STAAU LIPOPROTEIN SIGNAL PEPTIDASE (EC 3.4.23.36) PROLIPOPROTEIN SIGNAL PEPTIDASE (SIGNAL PEPTIDASE II) (SPASE II). 221 7 2524 3468 gi.vertline.1353527 ORF10 [*Bacteriophage rit*] 66 44 222 13 8272 8988 gi.vertline.466719 No definition line found [*Escherichia coli*] 223 18 15210 15971 gi.vertline.496520 orf iota [*Streptococcus pyogenes*] 66 57 232 5 3494 2715 gi.vertline.142706 comG1 gene product [*Bacillus subtilis*] 66 41 235 3 1774 734 gi.vertline.580897 OppB gene product [*Bacillus subtilis*] 66 47 244 2 906 1520 gi.vertline.15354 ORF 55.9 [*Bacteriophage T4*] 66 46 259 3 2355 1867 gi.vertline.56312 Gephyrin [*Rattus norvegicus*] 66 55 271 1 1 675 gi.vertline.1574748 tRNA pseudouridine 55 synthase (truB) 66 53 [*Haemophilus influenzae*] 277 1 1 927 gi.vertline.1303799 YqeN [*Bacillus subtilis*] 66 45 291 5 4587 3547 gnl.vertline.PID.vertline.e257609 sugar-binding transport protein 66 46 [*Anaerocellum thermophilum*] 292 25 20451 19912 gi.vertline.1649035 high-affinity periplasmic glutamine 66 50 binding protein [*Salmonella typhimurium*] 300 1 2302 77 gi.vertline.289262 comE ORF3 [*Bacillus subtilis*] 66 46 301 4 4290 3265 sp.vertline.P13226.vertline.GALE\_S- TR UDP-GLUCOSE 4-EPIMERASE (EC 5.1.3.2) 66 51 LI (GALACTOWALDENASE). 301 5 4516 4689 gnl.vertline.PID.vertline.e212-164 PSII, protein N [*Odontella sinensis*] 66 58 314 1 360 4 gi.vertline.467452 unknown [*Bacillus subtilis*] 66 43 15 4 2559 2209 gi.vertline.1653498 ABC transporter [*Synechocystis* sp.] 66 44 320 3 2406 1081 gnl.vertline.PID.vertline.e250352 unknown [*Mycobacterium tuberculosis*] 66 35 332 2 157 921 gi.vertline.1303875 YghB [*Bacillus subtilis*] 66 44 334 2 1001 3076 gi.vertline.1651660 DNA ligase [*Synechocystis* sp.] 66 48 338 1 2 616 gi.vertline.845686 ORF-27 [*Staphylococcus aureus*] 66 54 338 7 5011 5496 gi.vertline.912476 No definition line found [*Escherichia coli*] 341 5 1935 3107 gi.vertline.142538 aspartate aminotransferase [*Bacillus* sp.] 66 44 343 3 2548 2045 gnl.vertline.PID.vertline.e289147 similar to single strand binding protein 66 44 [*Bacillus subtilis*] 345 20 22093 22461 gi.vertline.1657795 dihydroneopterin aldolase 66 45 [*Methylobacterium extorquens*] 353 3 2621 2379 gnl.vertline.PID.vertline.e257628 ORF

[Lactococcus lactis] 66 52 365 4 5117 4779 gi.vertline.1742868 Mutator MutT protein (7,8-dihydro-8- 66 54 oxoguanine-triphosphatase) (8-oxo-dgtpase) (EC 3.6.1.-) (DGTP pyrophosphohydrolase). [Escherichia coli] 376 1 3 1076 gi.vertline.1778517 glycerol dehydrogenase homolog 66 45 [Escherichia coli] 394 7 5980 5648 gi.vertline.486358 ORF\_YKL202w [Saccharomyces cerevisiae] 66 38 421 4 1469 2539 gi.vertline.606375 ORF\_f345 [Escherichia coli] 66 48 475 6 3978 3763 gi.vertline.532547 ORF14 [Enterococcus faecalis] 66 48 491 8 7710 7081 gi.vertline.1000453 TreR [Bacillus subtilis] 66 49 526 1 392 3 gi.vertline.1750125 xylulose kinase [Bacillus subtilis] 66 49 552 6 6147 5917 gi.vertline.1432152 PTS antiterminator [Klebsiella oxytoca] 66 37 571 2 560 1153 gi.vertline.1773132 multidrug resistance-like ATP-binding 66 38 protein Mdl [Escherichia coli] 575 3 1075 539 gi.vertline.1651722 guanylate kinase [Synechocystis sp.] 66 48 608 2 631 113 gi.vertline.1213334 OrfX; hypothetical 22.5 KD protein 66 41 downstream of type IV prepilin leader peptidase gene; Method: conceptual translation supplied by author [Vibrio vulnificus] 640 1 877 2 sp.vertline.P50487.vertline.YCPX\_CLO HYPOTHETICAL PROTEIN IN CPE 5'REGION 66 36 PE (FRAGMENT) 734 1 2 343 gi.vertline.1653602 hypothetical protein [Synechocystis sp.] 66 43 802 1 2 292 gnl.vertline.PID.vertline.e280516 voltage-gated sodium channel [Mus 66 58 musculus] 812 2 343 531 gi.vertline.511075 ORF2 [Streptococcus agalactiae] 66 51 823 1 1 393 gi.vertline.1303843 YqfV [Bacillus subtilis] 66 42 891 1 82 402 gi.vertline.567769 ORF5; predicted protein shows similarity 66 52 to ATP-binding transport proteins AmiE and AmiF of Streptococcus pneumoniae; disruptulon of RF5 leads to aminopterin resistance [Streptococcus parasanguis] 66 52 5 6 2630 3154 gi.vertline.1303811 YqeU [Bacillus subtilis] 65 50 16 1 2 628 gi.vertline.1742303 Acyl carrier protein phosphodiesterase 65 43 (ACP phosphodiesterase) (fragment), [Escherichia coli] 18 6 3360 2518 gi.vertline.601880 rep protein [Bacillus borstelensis] 65 40 21 11 7933 7706 gi.vertline.1500521 M. jannaschii predicted coding region 65 32 MJ1623 [Methanococcus jannaschii] 23 20 13459 13881 gi.vertline.488430 alcohol dehydrogenase 2 [Entamoeba 65 43 histolytica] 23 25 15987 16178 gnl.vertline.PID.vertline.e248966 F32D8.5 [Caenorhabditis elegans] 65 50 27 2 526 302 gi.vertline.1001644 regulatory components of sensory 65 44 transduction system [Synechocystis sp.] 29 9 6770 5727 sp.vertline.P36672.vertline.PTTB\_ECO PTS SYSTEM, **TREHALOSE**-SPECIFIC IIBC 65 45 LI COMPONENT (EIIBC-TRE) (**TREHALOSE**- PERMEASE IIBC COMPONENT) (PHOSPHOTRANSFERASE ENZYME II, BC COMPONENT) (BC 2.7.1.69) (EI-TRE). 31 5 4611 5207 gi.vertline.171625 guanylate kinase [Saccharomyces 65 39 cerevisiae] 32 7 4085 3915 gi.vertline.150158 29 kD protein [Mycoplasma genitalium] 65 51 33 8 7396 7638 gi.vertline.1573421 protein translocation protein, low 65 26 temperature (secE) [Haemophilus influenzae] 35 1 2 499 gi.vertline.1737500 transcription antiterminator [Bacillus 65 40 stearothermophilus] 45 6 2537 3037 gi.vertline.511455 unknown [Coxiella burnetii] 65 37 46 3 1028 2254 gi.vertline.1001642 dGTP triphosphohydrolase [Synechocystis 65 43 sp.] 47 12 14524 14264 gi.vertline.150209 ORF 1 [Mycoplasma mycoides] 65 34 50 3 2866 2051 gi.vertline.1303830 YgfL [Bacillus subtilis] 65 40 57 11 12955 13332 gnl.vertline.PID.vertline.e254999 phenylalanyl-tRNA synthetase beta subunit 65 51 [Bacillus subtilis] 62 1 2 484 gi.vertline.1573470 H. influenzae predicted coding region 65 57 H10491 [Haemophilus influenzae] 68 1 49 282 gi.vertline.1573250 aspartate aminotransferase (aspC) 65 52 [Haemophilus influenzae] 72 2 567 1325 gi.vertline.466645 alternate name yhiD [Escherichia coli] 65 40 81 5 3711 2938 gi.vertline.1732200 PTS permease for mannose subunit IIPMan 65 43 [Vibria furnissii] 83 18 12506 12745

pir.vertline.D64042.vertline.D64042 ribosomal-protein-alanine 65 50  
 acetyltransferase (rimI) homolog - Haemophilus influenzae (strain Rd KW20)  
 100 38 28229 28032 gi.vertline.183075 glial fibrillary acidic protein [Homo 65  
 43 sapiens] 105 1 912 106 pir.vertline.S15248.vertline.YQBZCD fimC protein -  
 Dichelobacter nodosus 65 46 (serotype D) 106 5 6097 5102 gi.vertline.1143204  
 ORF2; Method: conceptual translation 65 44 supplied by author [Shigella  
 sonnei] 109 3 1165 899 gi.vertline.1573390 hypothetical [Haemophilus  
 influenzae] 110 7 5579 4257 pir.vertline.B44514.vertline.B44514  
 hypothetical protein 1 (vnfA 5' region) - 65 43 Azotobacter vinelandii] 120 3  
 1249 1632 sp.vertline.P54746.vertline.e.YBGB\_ECO HYPOTHETICAL PROTEIN IN HRSA  
 3'REGION 65 48 LI (FRAGMENT). 122 2 896 1654 gi.vertline.1335913 unknown  
 [Erysipelothrix rhusiopathiae] 65 48 145 4 2509 3210 gi.vertline.1208965  
 hypothetical 23.3 kd protein [Escherichia coli] 149 7 4407 3502  
 gi.vertline.145173 35 kDa protein [Escherichia coli] 65 46 154 8 5738 4926  
 gi.vertline.405804 transposase [Streptococcus thermophilis] 65 47 155 1 306  
 512 gi.vertline.285627 E.coli SecE homologous protein [Bacillus 65 48  
 subtilis] pir.vertline.S39858.vertline.S39858 secE protein homolog - Bacillus  
 subtilis sp.vertline.Q06799.vertline.SECE- \_BACSU PREPROTEIN TRANSLOCASE  
 SECE SUBUNIT. 158 1 150 1103 gi.vertline.289272 ferrichrome-binding protein  
 [Bacillus 65 40 subtilis] 158 16 14885 15946 gi.vertline.467172 add;  
 L308\_C2\_206 [Mycobacterium leprae] 65 36 173 4 2103 2912  
 gnl.vertline.PID.vertline.e254877 unknown [Mycobacterium tuberculosis] 65 41  
 173 12 9749 9054 gi.vertline.1652864 hypothetical protein [Synechocystis sp.]  
 65 50 179 16 15674 17035 gi.vertline.1171125 thioredoxin reductase  
 [Clostridium 65 41 litorale] 180 26 26911 28266  
 sp.vertline.P13692.vertline.P54\_ENTF P54 PROTEIN PRECURSOR. 65 39 C 193 6  
 2893 3795 gi.vertline.39787 adaA [Bacillus subtilis] 65 45 194 5 1843 2238  
 gi.vertline.47394 5-oxopropyl-peptidase [Streptococcus 65 48 pyogenes] 199 1  
 894 82 gi.vertline.1591118 nitrate transport ATP-binding protein 65 46  
 [Methanococcus jannaschii] 200 24 13441 13136 gi.vertline.144926 toxin A  
 [Clostridium difficile] 65 39 202 3 2925 1846 gi.vertline.413968 ipa-44d gene  
 product [Bacillus subtilis] 65 46 203 1 797 3 gi.vertline.1377832 unknown  
 [Bacillus subtilis] 65

#### Detail Description Table CWU - DETL:

[Azotobacter vinelandii] 60 31 34 4 6662 3279 gi.vertline.153952 polymerase  
 III polymerase subunit (dnaE) 60 37 [Salmonella typhimurium]  
 pir.vertline.A45915.vertline.A45915 DNA-directed DNA polymerase (EC 2.7.7.7)  
 III lpha chain - Salmonella typhimurium 39 1 47 466 gi.vertline.1561567  
 Unknown [Bacillus subtilis] 60 35 39 4 1855 1361 gi.vertline.298045 Orf154  
 [Streptomyces ambofaciens] 60 41 48 4 2554 4128 gi.vertline.1255259  
 o-succinylbenzoic acid (OSB) CoA ligase 60 40 [Staphylococcus aureus] 56 9  
 6682 5795 gi.vertline.413940 ipa-16d gene product [Bacillus subtilis] 60 40  
 65 3 2105 2593 gi.vertline.1573061 hypothetical [Haemophilus influenzae] 60 34  
 72 9 7854 8330 gi.vertline.606343 CG Site No. 28964 [Escherichia coli] 60 39  
 81 3 2053 1406 gi.vertline.1574770 phenylalanyl-tRNA synthetase beta-subunit  
 60 46 (pheT) [Haemophilus influenzae] 81 4 2987 2130 gi.vertline.147404  
 mannose permease subunit II-M-Man 60 34 [Escherichia coli] 81 12 8280 7150  
 gnl.vertline.PID.vertline.e254984 hypothetical protein [Bacillus subtilis] 60  
 44 83 22 16887 16537 gi.vertline.509672 repressor protein [Bacteriophage  
 Tuc2009] 60 33 89 1 698 60 gi.vertline.840838 hypothetical 21.7 kDa protein

in ftsY 5' 60 36 region [Pseudomonas aeruginosa] 89 12 12641 11856  
 gi.vertline.1377843 unknown [Bacillus subtilis] 60 40 89 17 18879 15844  
 gi.vertline.666069 orf2 gene product [Lactobacillus 60 37 leichmannii] 94 6  
 2281 3384 gi.vertline.468760 ORF334 [Rhizobium meliloti] 60 36 98 1 12 1970  
 gi.vertline.1652892 ABC transporter [Synechocystis sp.] 60 38 99 3 978 1460  
 gi.vertline.473955 DNA-binding protein [Lactobacillus sp.] 60 31 100 35 26818  
 26333 gi.vertline.347851 junctional sarcoplasmic reticulum 60 48 glycoprotein  
 [Oryctolagus unicolor] 100 45 30072 30449 gi.vertline.143547 Sin regulatory  
 protein (ttg start codon) 60 43 [Bacillus subtilis] gi.vertline.1303886 SinR  
 [Bacillus subtilis] 102 8 5923 6561 gi.vertline.1633572 Herpesvirus saimiri  
 ORF73 homolog 60 25 [Kaposi's sarcoma-associated herpes-like virus] 109 1  
 362 3 pir.vertline.S10655.vertline.S10655 hypothetical protein X - Pyrococcus  
 woesei 60 33 (fragment) 110 16 14806 14087  
 pir.vertline.JH0364.vertline.JH0364 hypothetical protein 176 (SAGP 5' region)  
 60 35 - Streptococcus pyogenes 110 20 18929 18414 gi.vertline.142450 ahrC  
 protein [Bacillus subtilis] 60 39 110 21 19124 19624 gi.vertline.142450 ahrC  
 protein [Bacillus subtilis] 60 40 111 1 289 2 gi.vertline.1256618 transport  
 protein [Bacillus subtilis] 60 31 122 7 5627 9589 gi.vertline.217191  
 5'-nucleotidase precursor [Vibrio 60 39 parahaemolyticus] 123 5 4390 3659  
 gi.vertline.1197667 vitellogenin [Anolis pulchellus] 60 27 123 20 18102 18407  
 gi.vertline.1303705 YrkF [Bacillus subtilis] 60 34 128 32 26229 25492  
 gi.vertline.1652485 hypothetical protein [Synechocystis sp.] 60 29 129 5 4421  
 6259 gi.vertline.1303853 YggF [Bacillus subtilis] 60 36 131 2 1112 2338  
 gi.vertline.699112 ugpC gene product [Mycobacterium leprae] 60 41 131 4 3194  
 4036 gi.vertline.296356 putative membrane transport protein 60 32  
 [Clostridium perfringens] pir.vertline.A56641.vertline.A56641 probable  
 membrane transport protein - Clostridium perfringens 131 8 6669 7901  
 gi.vertline.537054 2',3'-cyclic-nucleotide 2'- 60 40 phosphodiesterase  
 [Escherichia coli] pir.vertline.S56438.vertline.s56438 2',3'-cyclic-nucleotide  
 2-phosphodiesterase (EC .1.4.16) - Escherichia coli 133 11 9854 10240  
 gnl.vertline.PID.vertline.e249654 YneR [Bacillus subtilis] 60 37 138 7 6793  
 6263 gi.vertline.1486247 unknown [Bacillus subtilis] 60 48 146 4 2831 2328  
 gi.vertline.39979 P18 [Bacillus subtilis] 60 38 149 6 3504 3316  
 gi.vertline.145173 35 kDa protein [Escherichia coli] 60 47 154 5 2599 3558  
 gi.vertline.1773109 similar to S. typhimurium apbA 60 41 [Escherichia coli]  
 155 5 3061 4701 gi.vertline.388269 traC [Plasmid pAD1] 60 38 155 11 8565 8927  
 gi.vertline.1197460 MtfB [Escherichia coli] 60 39 158 10 11123 10032  
 gi.vertline.581809 tmbC gene product [Treponema pallidum] 60 39 165 7 6131  
 5700 gi.vertline.1439527 EIIA-man [Lactobacillus curvatus] 60 35 172 4 3169  
 3810 gi.vertline.1001342 hypothetical protein [Synechocystis sp.] 60 42 174 2  
 1574 762 gi.vertline.1045808 hypothetical protein (GB:U00021\_19) 60 35  
 [Mycoplasma genitalium] 181 7 4975 4460 gi.vertline.683584 shikimate kinase  
 [Lactococcus lactis] 60 33 183 6 2719 2955 gi.vertline.1146198 ferredoxin  
 [Bacillus subtilis] 60 37 189 2 3528 2221 gi.vertline.396301 matches PS00041:  
 Bacterial regulatory 60 35 proteins, araC family signature [Escherichia coli]  
 193 5 3121 2600 gi.vertline.39788 adaB [Bacillus subtilis] 60 49 195 11 4623  
 6569 gnl.vertline.PID.vertline.e250887 potential coding region [Clostridium  
 60 39 difficile] 202 2 1837 1607 gi.vertline.693939 membrane ATPase  
 [Halobacterium volcanii] 60 32 206 7 4794 3754 gi.vertline.1574702 hypothetical  
 [Haemophilus influenzae] 60 42 209 2 1308 433  
 pir.vertline.A38587.vertline.A38587 collagen, corneal - chicken (fragment) 60  
 51 220 3 4263 1213 gi.vertline.437706 alternative truncated translation  
 product 60 41 from E.coli [Streptococcus pneumoniae] 222 9 6019 6522

gi.vertline.882463 protein-N(pi)-phosphohistidine-sugar 60 47  
 phosphotransferase [Escherichia coli] 222 12 8001 8336 gi.vertline.537035  
 ORF\_o101 [Escherichia coli] 60 33 233 2 1294 827 gi.vertline.145091  
 flavodoxin [Desulfovibrio salexigens] 60 39 242 11 7370 7627  
 gi.vertline.1353404 cytochrome oxidase subunit I [Metridium 60 28 senile] 249  
 3 1109 1768 gi.vertline.143156 membrane bound protein [Bacillus subtilis] 60  
 41 251 3 4053 1933 gi.vertline.1235662 RfbC [Myxococcus xanthus] 60 42 256 4  
 2614 3867 gi.vertline.532612 ecotropic retrovirus receptor [Mus 60 37  
 musculus] 260 2 1539 802 gi.vertline.1208447 metahloprotease transporter  
 [Serratia 60 35 marcescens] 261 5 4528 3179  
 gnl.vertline.PID.vertline.e246728 histidine kinase [Streptococcus gordonii] 60  
 25 269 3 2723 1563 gi.vertline.1591618 M. jannaschii predicted coding region  
 60 39 MJ0951 [Methanococcus jannaschii] 269 4 3541 2780 gi.vertline.1303794  
 YgeM [Bacillus subtilis] 60 36 269 11 7164 6595 gi.vertline.1303787 YgeG  
 [Bacillus subtilis] 60 38 271 2 677 1651 gnl.vertline.PID.vertline-.e269877  
 riboflavin kinase [Bacillus subtilis] 60 43 271 3 1639 2247  
 gi.vertline.537148 ORF\_f181 [Escherichia coli] 60 41 271 18 13502 13762  
 pir.vertline.S3934.vertline.S39341 grpE protein - Lactococcus lactis 60 40  
 277 2 1662 979 gi.vertline.1773109 similar to S. typhimurium apbA 60 41  
 [Escherichia coli] 279 13 10627 9773 gi.vertline.290545 f270 [Escherichia  
 coli] 60 41 290 2 790 1695 gi.vertline.152886 elongation factor Ts (tsf)  
 [Spiroplasma 60 38 citri] 291 4 3571 2612 gnl.vertline.PID.vertline.e257610  
 sugar-binding transport protein 60 40 [Anaerocellum thermophilum] 295 3 1309  
 2094 gi.vertline.1000453 TreR [Bacillus subtilis] 60 37 301 15 11063 11344  
 gi.vertline.535274 ORF1 [Streptococcus thermophilus] 60 36 310 3 2903 1266  
 gi.vertline.809765 aspartate aminotransferase (AA 1-402) 60 44 [Sulfolobus  
 solfataricus] pir.vertline.S07088.vertline.S0708- 8 aspartate transaminase  
 (EC 2.6.1.1) - **Sulfolobus** solfataricus 316 2 319 119 bbs.vertline.115298  
 polyprotein(coat protein) [raspberry 60 28 ringspot virus RRV, Peptide, 1107  
 aa] [Raspberry ringspot virus] 320 4 3085 2483 gi.vertline.143002 proton  
 glutamate symnport protein [Bacillus 60 26 caldotenax]  
 pir.vertline.S26246.vertline.S26246 glutamate/aspartate transport protein -  
 Bacillus aldotenax 323 1 1 681 gi.vertline.1477486 transposase [Burkholderia  
 cepacia] 60 44 330 4 3361 4488 gi.vertline.1778517 glycerol dehydrogenase  
 homolog 60 48 [Escherichia coli] 356 3 2471 2205 gi.vertline.57633 neuronal  
 myosin heavy chain [Rattus 60 40 rattus] 362 5 2458 2925  
 gnl.vertline.PID.vertline.e255090 hypothetical protein [Bacillus subtilis] 60  
 36 364 4 4096 5349 gi.vertline.1657522 hypothetical protein [Escherichia  
 coli] 60 41 383 1 654 4 gn.vertline.PID.vertline.e288399 F56H6.k  
 [Caenorhabditis elegans] 60 39 383 2 2208 853 gi.vertline.143536 sigma factor  
 54 [Bacillus subtilis] 60 37 386 2 130 510 gi.vertline.1046053 hypothetical  
 protein (SP:P32049) 60 42 [Mycoplasma genitalium] 399 26 25892 27757  
 gi.vertline.895747 putative cel operon regulator [Bacillus 60 30 subtilis]  
 399 27 27721 28239 gi.vertline.146281 gut operon activator (gutM) [Escherichia  
 60 35 coli] 401 4 2081 3523 gi.vertline.142833 ORF2 [Bacillus subtilis] 60 36  
 405 2 1353 763 gi.vertline.633113 ORF3 [Streptococcus sobrinus] 60 42 407 7  
 4380 4589 gi.vertline.1674126 (AE000043) Mycoplasma pneumoniae, MG280 60 39  
 homolog, from M. genitalium [Mycoplasma pneumoniae] 408 1 12 539  
 gi.vertline.455006 orf6 [Rhodococcus fascians] 60 42 421 7 4113 3925  
 gi.vertline.60020 ORF31 (AA1-868) [Human herpesvirus 3] 60 43

PGPUB-DOCUMENT-NUMBER: 20020102696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102696 A1

TITLE: RECOMBINANT THERMOSTABLE ENZYME WHICH FORMS NON-REDUCING  
SACCHARIDE  
FROM REDUCING AMYLACEOUS SACCHARIDE

PUBLICATION-DATE: August 1, 2002

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APPL-NO: 09/ 419305

DATE FILED: October 15, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued  
prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09419305 A1 19991015 parent continuation-of 08505448 19950721 US PATENTED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	190183/1994	1994JP-190183/1994	July 21, 1994
JP	189706/1995	1995JP-189706/1995	July 4, 1995

US-CL-CURRENT: 435/200,435/183

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pl of about 5.4-6.4, and forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

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Abstract Paragraph - ABTX:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pI of about 5.4-6.4, and forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

Summary of Invention Paragraph - BSTX:

[0002] The present invention relates to a recombinant enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3.

Summary of Invention Paragraph - BSTX:

[0004] Trehalose is a disaccharide which consists of 2 glucose molecules that are linked together with their reducing groups, and, naturally, it is present in fungi, algae, insects, etc., in an extremely small quantity. Having no reducing residue within the molecule, trehalose does not cause an unsatisfactory browning reaction even when heated in the presence of amino acids or the like, and because of this it can advantageously sweeten food products without fear of causing unsatisfactory coloration and deterioration. Trehalose, however, could not have been readily prepared in a desired amount by conventional production methods, so that it has not scarcely been used for sweetening food products.

Summary of Invention Paragraph - BSTX:

[0005] Conventional production methods are roughly classified into 2 groups, i.e. the one using cells of microorganisms and the other using a multi-enzymatic system where several enzymes are allowed to act on saccharides. The former, as disclosed in Japanese Patent Laid-Open No.154,485/75, is a method which comprises growing microorganisms such as bacteria and yeasts in nutrient culture media, and collecting trehalose mainly from the proliferated cells. The latter, as disclosed in Japanese Patent Laid-Open No.216,695/83, is a method which comprises providing maltose as a substrate, allowing a multi-enzymatic system using maltose- and trehalose-phosphorylases to act on maltose, and recovering the formed trehalose from the reaction system. The former facilitates the growth of microorganisms, but has a demerit that the content in the microorganisms is at most 15 w/w %, on a dry solid basis (d.s.b.). Although the latter can readily separate trehalose, it is

theoretically difficult to increase the trehalose yield by allowing such enzymes to act on substrates at a considerably-high concentration because the enzymatic reaction in itself is an equilibrium reaction of 2 different types of enzymes and the equilibrium point constantly inclines to the side of forming glucose phosphate.

#### Summary of Invention Paragraph - BSTX:

[0006] In view of the foregoing, the present inventors energetically screened enzymes which form non-reducing saccharides having a trehalose structure from amylaceous saccharides having a degree of glucose polymerization of at least 3, and have found that microorganisms such as those of the genera *Rhizobium* and *Arthrobacter* produce an absolutely novel enzyme which forms such non-reducing saccharides from such reducing amylaceous saccharides. They disclosed such an enzyme in Japanese Patent Application No.349,216/93. They also found that trehalose is readily formed from such non-reducing saccharides when glucoamylase or .alpha.-glucosidase acts on them.

#### Summary of Invention Paragraph - BSTX:

[0007] It was found that the enzymes produced from the aforesaid microorganisms have an optimum temperature of about 40.degree. C., and have some difficulties in their thermostability when used to prepare trehalose. It is recognized in this field that the recommendable temperature in the saccharification reaction of starch or amylaceous saccharides is one which exceeds 55.degree. C. because the contamination of microorganisms will occur at a temperature of 55.degree. C. or lower, decrease the pH of the reaction mixtures, and inactivate the enzymes used. Thus, a relatively-large amount of substrates remain intact. While the use of enzymes with a poor thermostability, a great care should be taken to control the pH, and, when the pH level lowers to extremely low level, alkalis should be added to reaction mixtures to increase the pH level as quickly as possible.

#### Summary of Invention Paragraph - BSTX:

[0008] In view of the foregoing, the present inventors screened thermostable enzyme with such a novel enzyme activity and have found that enzymes produced from microorganisms of the genus *Sulfolobus* including *Sulfolobus acidocaldarius* (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently produce such non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides. These micro-organisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large scale culture to industrially produce non-reducing saccharides having a trehalose structure as an end unit.

#### Summary of Invention Paragraph - BSTX:

[0010] It is an object of the present invention to provide a recombinant

thermostable enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides with a degree of glucose polymerization of at least 3 by using the recombinant DNA technology.

Summary of Invention Paragraph - BSTX:

[0015] It is another object of the present invention to provide a method for converting reducing amylaceous saccharides with a degree of glucose polymerization of at least 3 into non-reducing saccharides having a trehalose structure as an end unit.

Summary of Invention Paragraph - BSTX:

[0018] Forming non-reducing saccharides having a trehalose structure as an end unit from reducing saccharides having a degree of glucose polymerization of at least 3;

Summary of Invention Paragraph - BSTX:

[0029] The sixth object of the present invention is attained by a method for enzymatically converting reducing amylaceous saccharides which contains a step of allowing the recombinant thermostable enzyme to act on reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 to form non-reducing saccharides having a trehalose structure as an end unit.

Brief Description of Drawings Paragraph - DRTX:

[0030] FIG. 1 is a figure of the optimum temperature of a thermostable enzyme produced from Sulfolobus acidocaldarius (ATCC 33909).

Brief Description of Drawings Paragraph - DRTX:

[0031] FIG. 2 is a figure of the optimum pH of a thermostable enzyme produced from Sulfolobus acidocaldarius (ATCC 33909).

Brief Description of Drawings Paragraph - DRTX:

[0032] FIG. 3 is a figure of the thermostability of a thermostable enzyme produced from Sulfolobus acidocaldarius (ATCC 33909).

Brief Description of Drawings Paragraph - DRTX:

[0033] FIG. 4 is a figure of the pH stability of a thermostable enzyme produced from Sulfolobus acidocaldarius (ATCC 33909).

Detail Description Paragraph - DETX:

[0036] The recombinant thermostable enzyme according to the present invention forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 without inactivation even when allowed to react at a temperature exceeding 55.degree. C.

Detail Description Paragraph - DETX:

[0041] The present conversion method readily converts reducing amylaceous saccharide having a degree of glucose polymerization of at least 3 into non-reducing saccharides having a trehalose structure as an end unit.

Detail Description Paragraph - DETX:

[0042] The present invention has been accomplished based on the finding of a novel enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

Detail Description Paragraph - DETX:

[0044] Forming non-reducing saccharides having a trehalose structure as an end unit from reducing saccharides having a degree of glucose polymerization of at least 3;

Detail Description Paragraph - DETX:

[0057] The followings are experiments which were conducted to reveal the physicochemical properties of a thermostable enzyme produced from Sulfolobus acidocaldarius (ATCC 33909):

Detail Description Paragraph - DETX:

[0060] Into 500-ml flasks were put 100 ml aliquots of a liquid culture medium containing 0.1 w/v % polypeptone, 0.1 w/v % yeast extract, 0.2 w/v % ammonium sulfate, 0.05 w/v % potassium dihydrogen phosphate, 0.02 w/v % magnesium sulfate heptahydrate, 0.02 w/v % potassium chloride, and water, and the flasks were sterilized by autoclaving at 120.degree. C. for 20 min. After cooling the flasks a seed culture of Sulfolobus acidocaldarius (ATCC 33909) was inoculated into each liquid culture medium in each flask, followed by the incubation at 75.degree. C. for 24 hours under a rotary shaking condition of 130 rpm to

obtain a first seed culture. About 5 L of a fresh preparation of the same liquid culture medium was put in a 10-L fermenter, sterilized similarly as above, cooled to 75.degree. C., and adjusted to a pH 3.0, followed by inoculating one v/v % of the first seed culture into the sterilized liquid culture medium in the fermenter, and culturing the microorganisms at 75.degree. C. for 24 hours under an aeration condition of 500 ml/min. Thereafter, about 250 L of a fresh preparation of the same liquid culture medium was placed in a 300-L fermenter, sterilized similarly as above, cooled to 75.degree. C., and adjusted to a pH 3.0, followed by inoculating one v/v % of the second seed culture into the sterilized liquid culture medium, and culturing the microorganisms at 75.degree. C. for 42 hours under an aeration condition of 100 L/min.

Detail Description Paragraph - DETX:

[0071] The results in Table 1 show that the purified enzyme acted on reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 such as maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose to form non-reducing saccharides having a trehalose structure as an end unit such as .alpha.-glucosyltrehalose, .alpha.-maltosyltrehalose, .alpha.-maltotriosyltrehalose, .alpha.-maltotetraosyltrehalose and .alpha.-maltopentaosyltrehalose. In addition to these non-reducing saccharides and intact substrates, glucose and low molecular weight maltooligosaccharides as estimable hydrolysates of the substrates, were detected in the reaction mixtures, and this indicates that the purified enzyme has a hydrolytic activity. The yields of the non-reducing saccharides and hydrolysates from the substrates were respectively 30.2% and 27.6% for maltotriose, 65.4% and 18.4% for maltotetraose, and about 74-75% and about 2-3% for maltopentaose, maltohexaose and maltoheptaose. The purified enzyme formed non-reducing saccharides from maltooligosaccharides having a degree of glucose polymerization of at least 5 in a satisfactory yield, and less hydrolyzed the substrates, but did not newly form any saccharide from glucose and maltose.

Detail Description Paragraph - DETX:

[0097] A chromosomal DNA of Sulfolobus acidocaldarius (ATCC 33909) was screened by using an oligonucleotide as a probe which had been chemically synthesized based on the partial amino acid sequences in SEQ ID NOs:3 and 4, and this yielded a DNA fragment having a base sequence from the 5'-terminus consisting of about 2,200 base pairs in SEQ ID NO:2. The base sequence of the thermostable enzyme was decoded and revealing that it consists of 720 amino acids and has a partial amino acid sequence from the N-terminal in SEQ ID NO:1.

Detail Description Paragraph - DETX:

[0108] To 500-ml flasks were placed about 100 ml aliquots of a liquid culture medium consisting of 0.1 w/v % polypeptone, 0.1 w/v % yeast extract, 0.2 w/v % ammonium sulfate, 0.05 w/v % potassium dihydrogen phosphate, 0.02 w/v % magnesium sulfate heptahydrate, 0.02 w/v % potassium chloride, and water, and the flasks were sterilized by autoclaving at 120.degree. C. for 20 min,

cooled, and adjusted to a pH 3.0 by the addition of sulfate. A seed culture of **Sulfolobus acidocaldarius** (ATCC 33909) was inoculated into each flask, incubated at 75.degree. C. for 24 hours under a rotary shaking condition of 130 rpm to obtain a seed culture. About 5 L of a fresh preparation of the same liquid nutrient culture medium was placed in a 10-L fermenter, sterilized similarly as above, cooled to 75.degree. C., adjusted to a pH 3.0, and inoculated with one v/v % of the seed culture, followed by the incubation at 75.degree. C. for 24 hours under an aeration condition of 500 ml/min.

Detail Description Paragraph - DETX:

[0120] As a control, a seed culture of Escherichia coli XLI-Blue strain or **Sulfolobus acidocaldarius** (ATCC 33909) was inoculated into a fresh preparation of the same liquid culture medium but free of ampicillin. In the case of culturing **Sulfolobus acidocaldarius** (ATCC 33909), it was cultured and treated similarly as above except that the initial pH of the nutrient culture medium and the culturing temperature were respectively set to 3.0 and 75.degree. C. Assaying the resultant enzymatic activity, one L culture of **Sulfolobus acidocaldarius** (ATCC 33909) yielded about 1.8 units of the thermostable enzyme, and the yield was significantly lower than that of transformant ST35. Escherichia coli XLI-Blue strain used as a host did not form the thermostable enzyme.

Detail Description Paragraph - DETX:

[0121] Thereafter, the recombinant thermostable enzyme produced by the transformant ST35 was purified similarly as in Experiments 1 and 2 and examined for properties and features and revealing that it has substantially the same physicochemical properties of the thermostable enzyme from **Sulfolobus acidocaldarius** (ATCC 33909) because (i) the recombinant thermostable enzyme has a molecular weight of about 69,000-79,000 daltons on SDS-PAGE and an isoelectric point of about 5.4-6.4 on isoelectrophoresis, and (ii) it is not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min. These results indicate that the present thermostable enzyme can be prepared by the recombinant DNA technology with a significantly improved yield.

Detail Description Paragraph - DETX:

[0126] Analyses of the DNA fragments separated on the radiogram revealed that the complementary chain DNA contains the base sequence consisting of 2,200 base pairs in SEQ ID NO:5. An amino acid sequence that could be estimated from the base sequence was in SEQ ID NO:5, and it was compared with the partial amino acid sequences in SEQ ID NOs:3 and 4, and revealing that the partial amino acid sequence in SEQ ID NO:3 corresponded to that positioning from 1 to 30 in SEQ ID NO:5, and that in SEQ ID NO:4 corresponded to that positioning from 468 to 478 in SEQ ID NO:5. These results indicate that the present recombinant thermostable enzyme has the amino acid sequence from the N-terminal in SEQ ID NO:1, and, in the case of the DNA derived from **Sulfolobus acidocaldarius** (ATCC 33909), the amino acid sequence is encoded by the base sequence from the

5'-terminus in SEQ ID NO:2.

Detail Description Paragraph - DETX:

[0127] As is explained in the above, the thermostable enzyme, which forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3, was found as a result of the present inventors' long-term research. The thermostable enzyme has distinct physicochemical properties from those of other conventional enzymes. The present invention is to produce the thermostable enzyme by using the recombinant DNA technology. The present recombinant thermostable enzyme, as well as its preparation and uses, will be explained in detail with reference to the later described Examples.

Detail Description Paragraph - DETX:

[0128] The recombinant thermostable enzyme as referred to in the present invention means thermostable enzymes in general which are preparable by the recombinant DNA technology and capable of forming non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Generally, the recombinant thermostable enzyme according to the present invention has a revealed amino acid sequence, and, as an example, the amino acid sequence from the N-terminal as shown in SEQ ID NO:1, and homologous ones to it can be mentioned. Variants having amino acid sequences homologous to the one in SEQ ID NO:1 can be obtained by replacing one or more bases in SEQ ID NO:1 with other bases without substantially alternating the inherent physicochemical properties. Although even when used the same DNA and it also depends on hosts into which the DNA is introduced, the ingredients and components of nutrient culture media for culturing transformants, and their cultivation temperature and pH, there may be produced modified enzymes which have the inherent physicochemical properties but defect one or more amino acids in SEQ ID NO:1, or those which have one or more amino acids added newly to the N-terminal after the DNA expression as the result of the modification of intracellular enzymes of the hosts. Such variants can be used in the present invention as long as they have the desired physicochemical properties.

Detail Description Paragraph - DETX:

[0130] The DNA usable in the present invention includes those are derived from natural resources and those which are artificially synthesized as long as they have the aforesaid base sequences. The natural resources for the DNA according to the present invention are, for example, microorganisms of the genus **Sulfolobus** such as **Sulfolobus acidocaldarius** (ATCC 33909), and from which genes containing the present DNA can be obtained. The aforementioned microorganisms can be inoculated in nutrient culture media and cultured for about 1-3 days under aerobic conditions, and the resultant cells were collected from the cultures and subjected to ultrasonication or treated with a cell-wall lysis enzyme such as lysozyme or .beta.-glucanase to extract genes containing the present DNA. In this case, a proteolytic enzyme such as protease can be used

along with the cell-wall lysis enzyme, and, when treated the cells with an ultrasonic disintegrator, they may be treated in the presence of a surfactant such as sodium dodecyl sulfate (SDS) or with freezing and thawing method. The objective DNA is obtainable by treating the resultant with phenol extraction, alcohol sedimentation, centrifugation, protease treatment and/or ribonuclease treatment generally used in this field. To artificially synthesize the present DNA, it can be chemically synthesized by using the base sequence in SEQ ID NO:2, or can be obtained in a plasmid form by inserting a DNA, which encodes the amino acid sequence in SEQ ID NO:1, into an appropriate self-replicable vector to obtain a recombinant DNA, introducing the recombinant DNA into an appropriate host to obtain a transformant, culturing the transformant, separating the proliferated cells from the resultant culture, and collecting plasmids containing the objective DNA from the cells.

#### Detail Description Paragraph - DETX:

[0133] The recombinant DNA thus obtained can be introduced into appropriate host microorganisms including *Escherichia coli* and those of the genus *Bacillus* as well as actinomyces and yeasts. In the case of using *Escherichia coli* as a host, the DNA can be introduced thereinto by culturing the host in the presence of the recombinant DNA and calcium ion, while in the case of using a microorganism of the genus *Bacillus* as a host the competent cell method and the colony hybridization method can be used. Desired transformants can be cloned by the colony hybridization method or by culturing a variety of transformants in nutrient culture media containing reducing amylaceous saccharides having a degree of glucose polymerization of at least 3, and selecting the objective transformants which form non-reducing saccharides having a trehalose structure as an end unit from the reducing amylaceous saccharides.

#### Detail Description Paragraph - DETX:

[0134] The transformants thus obtained intra- and extra-cellularly produce the objective enzyme when cultured in nutrient culture media. Generally, liquid culture media in general supplemented with carbon sources, nitrogen sources and minerals, and, if necessary, further supplemented with small amounts of amino acids and vitamins can be used in the invention. Examples of the carbon sources are saccharides such as unprocessed starch, starch hydrolysate, glucose, fructose, sucrose and trehalose. Examples of the nitrogen sources are organic- and inorganic-substances containing nitrogen such as ammonia and salts thereof, urea, nitrate, peptone, yeast extract, defatted soy bean, corn steep liquor, and beef extract. Cultures containing the objective enzyme can be prepared by inoculating the transformants into nutrient culture media, and incubating them at a temperature of 20-65.degree. C. and a pH of 2-9 for about 1-6 days under aerobic conditions by the aeration-agitation method. Such cultures can be used intact as a crude enzyme, and, usually, cells in the cultures may be disrupted prior to use with ultrasonic and/or cell-wall lysis enzymes, followed by separating the thermostable enzyme from intact cells and cell debris by filtration and/or centrifugation and purifying the enzyme. The methods to purify the enzyme include conventional ones in general. From cultures intact cells and cell debris are eliminated and subjected to one or more methods such as concentration, salting out, dialysis, separatory



sedimentation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectric point electrophoresis.

#### Detail Description Paragraph - DETX:

[0135] As is described above, the recombinant thermostable enzyme according to the present invention has a specific feature of forming non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 even when allowed to act on at a temperature exceeding 55.degree. C. The formed non-reducing saccharides have a satisfactorily mild and high-quality sweetness as well as an adequate viscosity and moisture-retaining ability, and, as a great advantageous feature, they can sweeten food products without fear of causing unsatisfactory coloration and deterioration because they have no reducing residue within their molecules. With these features a variety of amylaceous saccharides, which have been put aside because of their reducibilities, can be converted into saccharides which have a satisfactory handleability, usefulness, and no substantial reducibility or extremely-reduced reducibility.

#### Detail Description Paragraph - DETX:

[0137] In the enzymatic conversion method according to the present invention, the present recombinant thermostable enzyme is generally allowed to coexist in an aqueous solution containing one or more of the above reducing amylaceous saccharides as a substrate, followed by the enzymatic reaction at a prescribed temperature and pH until a desired amount of the objective reducing amylaceous saccharides is formed. Although the enzymatic reaction proceeds even below a concentration of 0.1 w/w %, d.s.b., of a substrate, a concentration of 2 w/w % or higher, d.s.b., preferably, in the range of 5-50 w/w %, d.s.b., of a substrate can be satisfactorily used when used the present conversion method in an industrial-scale production. The temperature and pH used in the enzymatic reaction are set to within the range of which does not inactivate the recombinant thermostable enzyme and allows the enzyme to effectively act on substrates, i.e. a temperature of higher than 55.degree. C. but not higher than 85.degree. C., preferably, a temperature in the range of about 56-70.degree. C., and a pH of 4-7, preferably, a pH in the range of about 5-6. The amount and reaction time suitable for the present recombinant thermostable enzyme are chosen depending on the enzymatic reaction condition. Thus, the present recombinant thermostable enzyme converts reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 into non-reducing saccharides having a **trehalose** structure as an end unit, e.g. the conversion rate reaches up to about 74% when acts on maltopentaose.

#### Detail Description Paragraph - DETX:

[0139] The non-reducing saccharides thus obtained have a wide applicability to a variety of products which are apt to be readily damaged by the reducibility of saccharide sweeteners: For example, they can be satisfactorily used in food

products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant. Since the non-reducing saccharides almost qualitatively form trehalose when received an enzymatic action of a trehalose-releasing enzyme as disclosed in Japanese Patent Application No.79,291/94, they can be used as an intermediate for producing trehalose which could not have been readily prepared.

Detail Description Paragraph - DETX:

[0152] The purified enzyme was assayed for properties and features by the method in Experiment 2 and revealing that it had a molecular weight of about 69,000-79,000 daltons on SDS-PAGE and a pI of about 5.4-6.4 on isoelectrophoresis, and was not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min. These physicochemical properties were substantially the same as those of the enzyme from a donor microorganism of Sulfolobus acidocaldarius (ATCC 33909).

Detail Description Paragraph - DETX:

[0158] The product had a low DE of 4.8 and contained 12.8 w/w % .alpha.-glucosyltrehalose, 11.5 w/w % .alpha.-maltosyltrehalose, 46.6 w/w % .alpha.-maltotriosyltrehalose, 2.3 w/w % .alpha.-maltotetraosyltrehalose and 3.4 w/w % .alpha.-maltopentaosyl-trehalose, d.s.b. Similarly as the product in Example B-1, the product has a mild and moderate sweetness and an adequate viscosity and moisture-retaining ability, and can be satisfactorily used in compositions in general such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

Detail Description Paragraph - DETX:

[0166] Conversion into Powdery Product Containing Crystalline Trehalose

Detail Description Paragraph - DETX:

[0167] Forty parts by weight of "PINE-DEX #4", a reducing amylaceous saccharide produced by Matsutani Chemical Ind., Co., Ltd., Kyoto, Japan, was dissolved in 60 parts by weight of water, and the solution was heated to 65.degree. C., adjusted to pH 5.5, and admixed with one unit/g reducing amylaceous saccharide, d.s.b., of a recombinant thermostable enzyme obtained by the method in Example A-1, followed by the enzymatic reaction for 96 hours. The reaction mixture was heated at 97.degree. C. for 30 min to inactivate the remaining enzyme, diluted up to a concentration of about 20 w/w %, d.s.b., and admixed with 10 units/g reducing amylaceous saccharide, d.s.b., of "GLUCOZYME", a glucoamylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, followed by the enzymatic reaction for 40 hours. Thereafter, the reaction mixture was heated to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolorized with an activated charcoal, desalted and purified with an ion exchanger, and concentrated into an about 60 w/w % solution. The concentrate

with a trehalose content of 30.1 w/w %, d.s.b., was subjected to column chromatographic fractionation similarly as in Example B-2 except that "CG6000", a strong-acid cation exchange resin in Na<sup>+</sup>-form commercialized by Japan Organo Co., Ltd., Tokyo, Japan, was used to obtain a fraction containing about 97 w/w % trehalose, d.s.b.

#### Detail Description Paragraph - DETX:

[0168] The fraction was concentrated up to about 75 w/w %, d.s.b., transferred to a crystallizer, and gradually cooled while stirring to obtain a massecuite with a crystallization percentage of about 45 w/w %, d.s.b. The massecuite was sprayed downward from a nozzle equipped on the upper part of a spraying tower at a pressure of about 150 kg/cm<sup>sup.2</sup> while an about 85.degree. C. hot air was blowing downward from the upper part of the spraying tower, and the formed crystalline powder was collected on a wire-netting conveyer provided on the basement of the drying tower and gradually conveyed out of the spraying tower while an about 45.degree. C. hot air was blowing to the crystalline powder from under the conveyer. The crystalline powder thus obtained was transferred to an ageing tower and aged for 10 hours in a hot air stream to complete the crystallization and drying. Thus, a powdery hydrous crystalline trehalose was obtained in a yield of about 90 w/w % to the material, d.s.b.

#### Detail Description Paragraph - DETX:

[0174] As is described above, the present invention is based on the finding of a novel thermostable enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The present invention is to explore a way to produce such a thermostable enzyme in an industrial scale and in a relatively-high efficiency by the recombinant DNA technology. The present conversion method using the recombinant thermostable enzyme readily converts non-reducing amylaceous saccharides, having a degree of glucose polymerization of at least 3, into non-reducing saccharides having a trehalose structure as an end unit without fear of causing bacterial contamination. The non-reducing saccharides have a mild and high-quality sweetness, and, because they have no reducing residue within their molecules, they can be advantageously incorporated into compositions in general such as food products, cosmetics and pharmaceuticals without fear of causing unsatisfactory coloration and deterioration. The present recombinant thermostable enzyme is the one with a revealed amino acid sequence, so that it can be used freely in the preparations of non-reducing saccharides having a trehalose structure as an end unit which are premised to be used in food products and pharmaceuticals.

#### Claims Text - CLTX:

1. A purified recombinant thermostable enzyme having the following physicochemical properties: (1) Action Forming non-reducing saccharides having a trehalose structure as an end unit and having a degree of glucose polymerization of at least 3 from maltotetraose or reducing amylaceous saccharides having a degree of glucose polymerization of at least 3; (2)

Molecular weight About 69,000-79,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); (3) Isoelectric point (pI) About 5.4-6.4 on isoelectrophoresis; (4) Thermostability Substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min.; and (5) Amino acid sequence An amino acid sequence which is not identical to SEQ ID NO:1 but is a functional equivalent thereof, and which contains one or more amino acid residues selected partially from SEQ ID NO:3 and SEQ ID NO:4.

PGPUB-DOCUMENT-NUMBER: 20020090618

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020090618 A1

TITLE: Thermostable reverse transcriptases and uses thereof

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Smith, Michael D.	Rockville	MD	US	
Potter, Robert Jason	Frederick	MD	US	
Dhariwal, Gulshan	Potomac	MD	US	
Gerard, Gary F.	Frederick	MD	US	
Rosenthal, Kim	Laytonsville	MD	US	

APPL-NO: 09/ 845157

DATE FILED: May 1, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60207196 20000526 US

US-CL-CURRENT: 435/6,435/199 ,435/5

ABSTRACT:

The present invention is in the fields of molecular and cellular biology. The invention is generally related to reverse transcriptase enzymes and methods for the reverse transcription of nucleic acid molecules, especially messenger RNA molecules. Specifically, the invention relates to reverse transcriptase enzymes which have been mutated or modified to increase thermostability, decrease terminal deoxynucleotidyl transferase activity, and/or increase fidelity, and to methods of producing, amplifying or sequencing nucleic acid molecules (particularly cDNA molecules) using these reverse transcriptase enzymes or compositions. The invention also relates to nucleic acid molecules produced by these methods and to the use of such nucleic acid molecules to produce desired polypeptides. The invention also concerns kits comprising such enzymes or compositions.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Appl. No. 60/207,196, filed May 26, 2000, the entire disclosure of which is incorporated herein by reference.

----- KWIC -----

Detail Description Paragraph - DETX:

[0186] A variety of DNA polymerases are useful in accordance with the present invention. Such polymerases include, but are not limited to, *Thermus thermophilus* (Tth) DNA polymerase, *Thermus aquaticus* (Taq) DNA polymerase, *Thermotoga neapolitana* (Tne) DNA polymerase, *Thermotoga maritima* (Tma) DNA polymerase, *Thermococcus litoralis* (Tli or VENT.TM.) DNA polymerase, *Pyrococcus furiosus* (Pfu) DNA polymerase, *Pyrococcus* species GB-D (DEEPVENT.TM.) DNA polymerase, *Pyrococcus woosii* (Pwo) DNA polymerase, *Bacillus sterothennophilus* (Bst) DNA polymerase, *Bacillus caldophilus* (Bca) DNA polymerase, *Sulfolobus acidocaldarius* (Sac) DNA polymerase, *Thermoplasma acidophilum* (Tac) DNA polymerase, *Thermus flavus* (Tfl/Tub) DNA polymerase, *Thermus ruber* (Tru) DNA polymerase, *Thermus brockianus* (DYNAZYME.TM.) DNA polymerase, *Methanobacterium thermoautotrophicum* (Mth) DNA polymerase, *Mycobacterium* spp. DNA polymerase (Mtb, Mlep), and mutants, variants and derivatives thereof.

Detail Description Paragraph - DETX:

[0191] In addition to the enzyme components, the present compositions preferably comprise one or more buffers and cofactors necessary for synthesis of a nucleic acid molecule such as a cDNA molecule. Particularly preferred buffers for use in forming the present compositions are the acetate, sulfate, hydrochloride, phosphate or free acid forms of Tris-(hydroxymethyl)aminomethane (TRIS.RTM.), although alternative buffers of the same approximate ionic strength and pKa as TRIS.RTM. may be used with equivalent results. In addition to the buffer salts, cofactor salts such as those of potassium (preferably potassium chloride or potassium acetate) and magnesium (preferably magnesium chloride or magnesium acetate) are included in the compositions. Addition of one or more carbohydrates and/or sugars to the compositions and/or synthesis reaction mixtures may also be advantageous, to support enhanced stability of the compositions and/or reaction mixtures upon storage. Preferred such carbohydrates or sugars for inclusion in the compositions and/or synthesis reaction mixtures of the invention include, but are not limited to, sucrose, trehalose, glycerol, and the like. Furthermore, such carbohydrates and/or sugars may be added to the storage buffers for the enzymes used in the production of the enzyme compositions and kits of the invention. Such carbohydrates and/or sugars are commercially available from a number of sources, including Sigma (St. Louis, Mo.).

Detail Description Paragraph - DETX:

[0197] In another aspect, the compositions and reverse transcriptases of the invention may be prepared and stored in dry form in the presence of one or more carbohydrates, sugars, or synthetic polymers. Preferred carbohydrates, sugars or polymers for the preparation of dried compositions or reverse transcriptases include, but are not limited to, sucrose, trehalose, and polyvinylpyrrolidone (PVP) or combinations thereof. See, e.g., U.S. Pat. Nos. 5,098,893, 4,891,319, and 5,556,771, the disclosures of which are entirely incorporated

herein by reference. Such dried compositions and enzymes may be stored at various temperatures for extended times without significant deterioration of enzymes or components of the compositions of the invention. Preferably, the dried reverse transcriptases or compositions are stored at 4.degree. C. or at -20.degree. C.

PGPUB-DOCUMENT-NUMBER: 20020081581

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081581 A1

TITLE: COMPOSITIONS AND METHODS FOR REVERSE TRANSCRIPTION OF NUCLEIC  
ACID  
MOLECULES

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GERARD, GARY F.	FREDERICK	MD	US	
SMITH, MICHAEL D.	ROCKVILLE	MD	US	
CHATTERJEE, DEB K.	NORTH POTOMAC	MD	US	

APPL-NO: 09/ 245026

DATE FILED: February 5, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued  
prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09245026 A1 19990205 parent division-of 09064057 19980422 US PENDING  
non-provisional-of-provisional 60044589 19970422 US  
non-provisional-of-provisional 60049874 19970617 US

US-CL-CURRENT: 435/6,435/91.2

ABSTRACT:

The present invention is generally related to compositions and methods for the reverse transcription of nucleic acid molecules, especially messenger RNA molecules. Specifically, the invention relates to compositions comprising mixtures of polypeptides having reverse transcriptase (RT) activity, and to methods of producing, amplifying or sequencing nucleic acid molecules (particularly cDNA molecules) using these compositions or polypeptides, particularly at temperatures above about 55.degree. C. The invention also relates to nucleic acid molecules produced by these methods, to vectors and host cells comprising these nucleic acid molecules, and to the use of such nucleic acid molecules to produce desired polypeptides. The invention also relates to methods for producing Rous Sarcoma Virus (RSV) and Avian Myeloblastosis Virus (AMV) RTs or other Avian Sarcoma-Leukosis Virus (ASLV) RTs (.alpha. and/or .beta. subunits thereof), to isolated nucleic acid molecules encoding such RSV RT, AMV RT or other ASLV RT subunits, to vectors and host cells comprising these isolated nucleic acid molecules and to RSV RT, AMV RT



and other ASLV RT subunits produced by these methods. The invention further relates to nucleic acid molecules encoding recombinant heterodimeric RT holoenzymes, particularly heterodimeric RSV RTs, AMV RTs or other ASLV RTs (which may be .alpha..beta. RTs, .beta..beta. RTs, or .alpha. RTs), vectors (particularly baculovirus vectors) and host cells (particularly insect and yeast cells) comprising these nucleic acid molecules, methods for producing these heterodimeric RTs and heterodimeric RTs produced by these methods. The invention also relates to kits comprising the compositions, polypeptides, or RSV RTs, AMV RTs or other ASLV RTs of the invention.

#### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application Nos. 60/044,589, filed Apr. 22, 1997, and 60/049,874, filed Jun. 17, 1997, the disclosures of which are entirely incorporated by reference herein.

----- KWIC -----

#### Detail Description Paragraph - DETX:

[0115] A variety of DNA polymerases are useful in accordance with the present invention. Such polymerases include, but are not limited to, *Thermus thermophilus* (Tth) DNA polymerase, *Thermus aquaticus* (Taq) DNA polymerase, *Thermotoga neapolitana* (Tne) DNA polymerase, *Thermotoga maritima* (Tma) DNA polymerase, *Thermococcus litoralis* (Tli or VENT.TM.) DNA polymerase, *Pyrococcus furiosus* (Pfu) DNA polymerase, DEEPVENT.TM. DNA polymerase, *Pyrococcus woosii* (Pwo) DNA polymerase, *Bacillus stearothermophilus* (Bst) DNA polymerase, *Bacillus caldophilus* (Bca) DNA polymerase, *Sulfolobus acidocaldarius* (Sac) DNA polymerase, *Thermoplasma acidophilum* (Tac) DNA polymerase, *Thermus flavus* (Tfi/Tub) DNA polymerase, *Thermus ruber* (Tru) DNA polymerase, *Thermus brockianus* (DYNAZYME.TM.) DNA polymerase, *Methanobacterium thermoautotrophicum* (Mth) DNA polymerase, *Mycobacterium* spp. DNA polymerase (Mtb, Mlep), and mutants, variants and derivatives thereof.

#### Detail Description Paragraph - DETX:

[0121] In addition to the enzyme components, the present compositions preferably comprise one or more buffers and cofactors necessary for synthesis of a nucleic acid molecule such as a cDNA molecule. Particularly preferred buffers for use in forming the present compositions are the acetate, sulfate, hydrochloride, phosphate or free acid forms of Tris-(hydroxymethyl)aminomethane (TRIS.RTM.), although alternative buffers of the same approximate ionic strength and pKa as TRIS.RTM. may be used with equivalent results. In addition to the buffer salts, cofactor salts such as those of potassium (preferably potassium chloride or potassium acetate) and magnesium (preferably magnesium chloride or magnesium acetate) are included in the compositions. Addition of one or more carbohydrates and/or sugars to the compositions and/or synthesis reaction mixtures may also be advantageous, to support enhanced stability of the compositions and/or reaction mixtures upon storage. Preferred

such carbohydrates or sugars for inclusion in the compositions and/or synthesis reaction mixtures of the invention include, but are not limited to, sucrose, trehalose, and the like. Furthermore, such carbohydrates and/or sugars may be added to the storage buffers for the enzymes used in the production of the enzyme compositions and kits of the invention. Such carbohydrates and/or sugars are commercially available from a number of sources, including Sigma (St. Louis, Mo.).

#### Detail Description Paragraph - DETX:

[0127] In another aspect, the compositions and reverse transcriptases of the invention may be prepared and stored in dry form in the presence of one or more carbohydrates, sugars, or synthetic polymers. Preferred carbohydrates, sugars or polymers for the preparation of dried compositions or reverse transcriptases include, but are not limited to, sucrose, trehalose, and polyvinylpyrrolidone (PVP) or combinations thereof. See, e.g., U.S. Pat. Nos. 5,098,893, 4,891,319, and 5,556,771, the disclosures of which are entirely incorporated herein by reference. Such dried compositions and enzymes may be stored at various temperatures for extended times without significant deterioration of enzymes or components of the compositions of the invention. Preferably, the dried reverse transcriptases or compositions are stored at 4.degree. C. or at -20.degree. C.

#### Detail Description Paragraph - DETX:

[0186] Buffers. The pH of all buffers was determined at 23.degree. C., and buffers were stored at 4.degree. C. until use. Crack Buffer contained 50 mM Tris-HCl (pH 7.9), 0.5 M KCl, 0.02% (v/v) Triton X-100 and 20% (v/v) glycerol. Just before use, the following protease inhibitors (Boehringer Mannheim; Indianapolis, Ind.) were added to Crack Buffer at the final concentrations indicated: leupeptin (2 .mu.g/ml), Pefabloc (48 .mu.g/ml), pepstatin A (2 .mu.g/ml), benzamidine (800 .mu.g/ml) and PMSF (50 .mu.g/ml). Buffer A contained 20 mM Tris-HCl (pH 7.9), 0.25 M KCl, 0.02% (v/v) Triton X-100, and 10% (v/v) glycerol. Buffer B was Buffer A with 1 M imidazole added. Buffer S contained 50 mM Tris-HCl (pH 8.2), 0.02% (v/v) Triton X-100, 10% (v/v) glycerol, 0.1 mM EDTA and 1 mM dithiothreitol (DTT). Buffer T was Buffer S with 1 M KCl added. Buffer H contained 20 mM potassium phosphate (pH 7.1), 0.02% (v/v) Triton X-100, 20% (v/v) glycerol, 0.1 mM EDTA and 1 mM DTT. Buffer J was Buffer H with 1 M KCl added. Storage Buffer contained 200 mM potassium phosphate (pH 7.1), 0.05 % (v/v) NP-40, 50% glycerol (v/v), 0.1 mM EDTA, 1 mM DTT, and 10% (w/v) trehalose.

PGPUB-DOCUMENT-NUMBER: 20010024793

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010024793 A1

TITLE: Nucleic acid-free thermostable enzymes and methods of production thereof

PUBLICATION-DATE: September 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Goldstein, Adam S.	New Market	MD	US	
Hughes, A. John JR.	Germantown	MD	US	

APPL-NO: 09/ 866816

DATE FILED: May 30, 2001

RELATED-US-APPL-DATA:

child 09866816 A1 20010530 parent continuation-of 09229967 19990114 US GRANTED  
parent-patent 6245533 US child 09229967 19990114 US parent continuation-of  
08778082 19970102 US GRANTED parent-patent 5861295 US

US-CL-CURRENT: 435/6,435/183 ,435/194 ,435/91.1 ,435/91.2

ABSTRACT:

The present invention provides thermostable enzymes, such as DNA polymerases and restriction endonucleases, that are substantially free from contamination with nucleic acids. The invention also provides methods for the production of these enzymes, and kits comprising these enzymes which may be used in amplifying or sequencing nucleic acid molecules, including through use of the polymerase chain reaction (PCR).

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0006] These disruption approaches have several advantages, including their ability to rapidly and completely (in the case of physical methods) disrupt the bacterial cell such that the release of intracellular proteins is maximized. In fact, these approaches have been used in the initial steps of processes for the purification of a variety of bacterial cytosolic enzymes, including natural and recombinant proteins from mesophilic organisms such as *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Laurent, S. J., and Vannier, F.

S., *Biochimie* 59:747-750 (1977); Cull, M., and McHenry, C. S., *Meth. Enzymol.* 182:147-153 (1990); Hughes, A. J., Jr., et al., *J. Cell. Biochem. Suppl.* 0 16 (Part B):84 (1992); Ausubel, F. M., et al., in: *Current Protocols in Molecular Biology*, New York: John Wiley & Sons, pp. 4.4.1-4.4.7 (1993)), as well as phosphatases, restriction enzymes, DNA or RNA polymerases and other proteins from thermophilic bacteria and archaea such as *Thermus aquaticus*, *Thermus thermophilus*, *Thermus flavus*, *Thermus caldophilus*, *Thermotoga maritima*, and *Sulfolobus acidocaldarius* (Shinomiya, T., et al., *J. Biochem.* 92(6):1823-1832 (1982); Elie, C., et al., *Biochim. Biophys. Acta* 951(2-3):261-267 (1988); Palm, P., et al., *Nucl. Acids Res.* 21(21):4904-4908 (1993); Park, J. H., et al., *Eur. J. Biochem.* 214(1):135-140 (1993); Harrell, R. A., and Hand, R. P., *PCR Meth. Appl.* 3(6):372-375 (1994); Meyer, W., et al., *Arch. Biochem. Biophys.* 319(1):149-156 (1995)).

#### Detail Description Paragraph - DETX:

[0021] Thermostable enzymes (e.g., DNA polymerases or restriction enzymes) may be prepared according to the methods of the present invention from a variety of thermophilic bacteria that are available commercially (for example, from American Type Culture Collection, Rockville, Md.). Suitable for use as sources of thermostable enzymes are the thermophilic bacteria *Thermus aquaticus*, *Thermus thermophilus*, *Thermococcus litoralis*, *Pyrococcus furiosus*, *Pyrococcus woosii* and other species of the *Pyrococcus* genus, *Bacillus stearothermophilus*, *Sulfolobus acidocaldarius*, *Thermoplasma acidophilum*, *Thermus flavus*, *Thermus ruber*, *Thermus brockianus*, *Thermotoga neapolitana*, *Thermotoga maritima* and other species of the *Thermotoga* genus, and *Methanobacterium thermoautotrophicum*, and mutants of each of these species. It will be understood by one of ordinary skill in the art, however, that any thermophilic microorganism may be used as a source for preparation of thermostable enzymes according to the methods of the present invention. Bacterial cells may be grown according to standard microbiological techniques, using culture media and incubation conditions suitable for growing active cultures of the particular species that are well-known to one of ordinary skill in the art (see, e.g., Brock, T. D., and Freeze, H., *J. Bacteriol.* 98(1):289-297 (1969); Oshima, T., and Imahori, K, *Int. J. Syst. Bacteriol.* 24(1):102-112 (1974)).

#### Detail Description Paragraph - DETX:

[0037] Following their purification, the substantially DNA-free thermostable enzymes may be stored until use in a buffered solution at temperatures of about -80.degree. to 25.degree. C., most preferably at -80.degree. to 4.degree. C., or in lyophilized form. Alternatively, the enzymes may be stabilized by drying in the presence of a sugar such as trehalose (U.S. Pat. Nos. 5,098,893 and 4,824,938) or acacia gum, pectin, carboxymethylcellulose, carboxymethylhydroxyethylcellulose, guar, carboxy guar, carboxymethylhydroxypropyl guar, laminaran, chitin, alginates or carrageenan. In addition, the enzymes provided by the present invention may be directly formulated into compositions to be used in techniques requiring the use of thermostable enzymes, such as compositions for nucleic acid sequencing or amplification in the case of thermostable DNA polymerases such as Taq, Tne, or Tma DNA polymerases, or mutants, derivatives or fragments thereof. These

formulations may be concentrated solutions of the enzymes, or solutions of the enzymes at working concentrations which may comprise additional components and which may be prepared as described in co-pending U.S. patent application Ser. No. 08/689,815, by Ayoub Rashtchian and Joseph Solus, entitled "Stable Compositions for Nucleic Acid Sequencing and Amplification," filed Aug. 14, 1996, which is incorporated by reference herein in its entirety.

US-PAT-NO: 6465236

DOCUMENT-IDENTIFIER: US 6465236 B1

TITLE: Thermostable collagen-digesting enzyme, novel microorganism producing the enzyme and process for producing the enzyme

DATE-ISSUED: October 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nishino; Tokuzo	Sendai	N/A	N/A	JP
Nakayama; Toru	Sendai	N/A	N/A	JP
Tsuruoka; Naoki	Sendai	N/A	N/A	JP
Akai; Minoru	Sendai	N/A	N/A	JP

APPL-NO: 09/ 807745

DATE FILED: April 18, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	11-242816	August 30, 1999

PCT-DATA:

APPL-NO: PCT/JP99/06392  
DATE-FILED: November 16, 1999  
PUB-NO: WO01/16302  
PUB-DATE: Mar 8, 2001  
371-DATE: Apr 18, 2001  
102(E)-DATE: Apr 18, 2001

US-CL-CURRENT: 435/221; 435/219 ; 435/220 ; 435/252.1 ; 435/252.5

ABSTRACT:

Bacillus sp. NTAP-1 having been deposited under accession number FERM BP-6926; and a collagen-decomposing enzyme produced by bacterium. The above enzyme (1) has a capability of hydrolyzing, at the highest efficiency, collagen and gelatin from among casein, gelatin, albumin and collagen; (2) shows the optimum pH of 3.5 to 4.5; (3) shows the optimum temperature of 65 to 70.degree. C.; (4) after heating at 60.degree. C. at pH 6.0 for 4 hours, sustains an activity amounting to 60% or more of the level before the heat treatment; (5) remains stable at pH 3 to 6; and a molecular weight of approximately 46,000 when measured by SDS-PAGE.

4 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

Detailed Description Text - DETX:

Does not decompose glycelol, erythritol, adonitol, .beta.-methyl-D-xylose, galactose, mannose, rhamnose, dulcitol, .alpha.-methyl-D-mannose, .alpha.-methyl-D-glucose, N-acetyl-glucosamine, amidagline, arbutin, aesuculin, salicin, cellobiose, maltose, milk sugar, melibiose, cane sugar, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, gentiobiose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconic acid.

Detailed Description Text - DETX:

Among known species of the genus *Bacillus*, *B. acidocaldarius*, *B. lichenformis*, or *B. coagulans* are known to be thermophilic and acidophilic. However, this strain should not be *B. lichenformis* and *B. coagulans* because *B. lichenformis* and *B. coagulans* are catalase-positive and can grow at 40.degree. C. but not at 65.degree. C. Also, it is different from the standard species of *B. acidocaldarius* because it produces acetoin. Therefore, it is not possible to confirm that whether it is a modified species of *B. acidocaldarius* or it belongs to a different species; the species of this bacteria can not be specified.

US-PAT-NO: 6448049

DOCUMENT-IDENTIFIER: US 6448049 B1

TITLE: Starch conversion process

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsutsumi; Noriko	Chiba-ken	N/A	N/A	JP
Bisg.ang.rd-Frantzen;	Bagsv.ae	N/A	N/A	DK
Henrik	butted.rd	N/A	N/A	DK
Svensden; Allan	Birker.o			
	slashed.d			

APPL-NO: 09/ 544123

DATE FILED: April 6, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional of application Ser. No. 09/129,075 filed on Aug. 4, 1998, now U.S. Pat. No. 6,087,149 which is a continuation of PCT/DK98/00304 filed Jul. 2, 1998, and claims priority under 35 U.S.C. 119 of Danish application no. 0787/97 filed on Jul. 2, 1997, and U.S. application Ser. No. 60/055,567 filed Aug. 13, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0787/97	February 7, 1997

US-CL-CURRENT: 435/98; 435/210

ABSTRACT:

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus *Rhodothermus* and to cloned DNA sequences encoding isoamylases derived from a strain of *Rhodothermus* or *Sulfolobus*, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

4 Claims, 7 Drawing figures

Exemplary Claim Number: 1



Number of Drawing Sheets: 7

----- KWIC -----

Abstract Text - ABTX:

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus *Rhodothermus* and to cloned DNA sequences encoding isoamylases derived from a strain of *Rhodothermus* or *Sulfolobus*, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX:

The invention relates to a starch conversion process of the type which includes a debranching step. The invention also relates to the use of a thermostable isoamylase for debranching starch. The invention further relates to an isolated isoamylase obtained from a strain of the genus *Rhodothermus*, to cloned DNA sequences encoding isoamylases derived from a strain of *Rhodothermus* or *Sulfolobus*, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX:

Starches such as corn, potato, wheat, manioc and rice starch are used as the starting material in commercial large scale production of sugars, such as high fructose syrup, high maltose syrup, maltodextrins amylose, trehalose, G2-G8 oligosaccharides (including functional oligosaccharides) and other carbohydrate products such as fat replacers.

Drawing Description Text - DRTX:

FIG. 5 shows the pH curve of *Sulfolobus acidocaldarius* isoamylase.

Detailed Description Text - DETX:

Specific examples of thermostable debranching enzymes are the thermostable isoamylases derived from the thermophilic bacteria such as *Sulfolobus acidocaldarius* ATCC 33909 (Maruta, K. et al., *Biochimica et Biophysica Acta* 1291, p. 177-181 (1996)), *Sulfolobus solfataricus* ATCC 35092 (accession number: Y08256) and *Rhodothermus marinus* DSM 4252 as will be described further below.

Detailed Description Text - DETX:

Isoamylases which can be used according to the invention include the thermostable isoamylase derived from the thermophilic archaeobacteria **Sulfolobus acidocaldarius**, **Sulfolobus** solfataricus and the thermophilic eubacterium *Rhodothermus marinus* (as will be described in details below).

Detailed Description Text - DETX:

In the case of the starch conversion process is a starch depolymerization process the thermostable isoamylase is used in combination with an .alpha.-amylase during the liquefaction step. The thermostable isoamylase may be derived from the thermophilic archaeobacteria **Sulfolobus acidocaldarius** or **Sulfolobus** solfataricus or from *Rhodothermus marinus*.

Detailed Description Text - DETX:

The homology search showed that the most related known sequence(s) were isoamylases from **Sulfolobus acidocaldarius** and **Sulfolobus** solfataricus. The DNA sequence of the invention (SEQ ID NO: 3) encoding an isoamylase shows about 51-52% DNA homology to the known isoamylase sequences from **Sulfolobus acidocaldarius** and **Sulfolobus** solfataricus and the corresponding amino acid sequence of the isoamylase of the invention (SEQ ID NO: 4) shows about 54-55% homology to a deduced amino acid sequence based on the known DNA sequence above.

Detailed Description Text - DETX:

Cloning of a DNA Sequence from a Strain of **Sulfolobus**

Detailed Description Text - DETX:

The invention also relates to a cloned DNA sequence encoding an enzyme with isoamylase activity derived from a strain of **Sulfolobus**. The DNA sequence may be derived from a strain of **Sulfolobus acidocaldarius** or **Sulfolobus** solfataricus.

Detailed Description Text - DETX:

The sequences may be the DNA sequences encoding an isoamylase from **Sulfolobus acidocaldarius** disclosed in Biochim. Biophys. Acta, 1291, p.117-181 (1996), it may be the DNA sequence from **Sulfolobus** solfataricus available in GeneBank under the Accession no. Y08256.

Detailed Description Text - DETX:

Cloning of the Sulf. lobus acidocaldarius DNA sequence disclosed in Biochim. Biophys. Acta, 1291, p.177-181 (1996) is described below.

Detailed Description Text - DETX:

Cloning and Expression of Sulfolobus acidocaldarius Isoamylase Gene

Detailed Description Text - DETX:

The isoamylase gene from Sulfolobus acidocaldarius was cloned by PCR. The primers were designed based on the sequence shown in the article of Table 1. primer SINH: 5'-gtatatcaaagcttatgaaagatcgaccattaaagcctg-3' (SEQ ID NO:7) primer SICX: 5'-ggtgtctagatcactggaactctatcctcctgta-3' (SEQ ID NO:8)

Detailed Description Text - DETX:

Cloned gene from Sulfolobus acidocaldarius was inserted at Hind III and XbaI site of pJSOHX and expression vector named pFSI82 was obtained. pFSI 82 was transformed to Saccharomyces cerevisiae YNG318. pFSI82 map is shown in FIG. 4.

Detailed Description Text - DETX:

Characterization of the Cloned Sulfolobus acidocaldarius Isoamylase

Detailed Description Text - DETX:

The pH optimum of the Sulfolobus acidocaldarius isoamylase was determined. The Reaction was carried out at 70.degree. C. The checked pH was between pH 3.5-7.5. The pH optimum was determined to pH 5.5. The pH curve is shown in FIG. 5.

Detailed Description Text - DETX:

Temperature optimum of the Sulfolobus isoamylase was determined. The reaction was carried out at pH5.5 at between 40.degree.-90.degree. C. The optimum temperature was determined to be around 70.degree. C. The temperature curve is shown in FIG. 6.

Detailed Description Paragraph Table - DETL:

identity Origins of isoamylase genes DNA a.a. Pseudomonas amyloclavata\*1 50.4 % 33.0% Pseudomonas sp. SMP1\*2 50.4 % 33.0% Flavobacterium sp.\*3 54.0 % 34.1% Flavobacterium odoratum\*4 54.8 % 36.6% Sulfolobus acidocaldarius\*5 51.8

% 54.2% **Sulfolobus** solfataricus\*6 51.1 % 55.0% \*1: Table 1, 1 \*2: Table 1, 2  
 \*3: Table 1, 3 \*4: Table 1, 4 \*5: Table 1, 5 \*6: GenBank: Accession number;  
 Y08256

#### Detailed Description Paragraph Table - DETL:

TABLE 1 1 *Pseudomonas amyloclavata* Biochim. Biophys. Acta, 1087, p. 309-315  
 (1990) 2 *Pseudomonas* sp. European patent publication num- ber: EP 0 302 838  
 A2 3 *Flavobacterium* sp. International patent publication number: WO.96/03513  
 4 *Flavobacterium odoratum* Japanese patent publication num- ber: JP08023981-A  
 5 ***Sulfolobus acidocaldarius*** Biochim. Biophys. Acta, 1291, p. 177-181 (1996)

#### Detailed Description Paragraph Table - DETL:

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 Pro Glu Glu Gly 85 90 95 His Arg Phe Asn Pro Asn Lys Val Leu Leu Asp Pro Tyr  
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 Val Ala His Pro Ala Val Ile Ser His Leu Gln Lys Leu Gly Ile 100 105 110 Thr  
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 Arg Ser 20 25 30

US-PAT-NO: 6410241

DOCUMENT-IDENTIFIER: US 6410241 B1

TITLE: Methods of screening open reading frames to determine whether they encode polypeptides with an ability to generate an immune response

DATE-ISSUED: June 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sykes; Kathryn F.	Dallas	TX	N/A	N/A
Johnston; Stephen Albert	Dallas	TX	N/A	N/A

APPL-NO: 09/ 535366

DATE FILED: March 24, 2000

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999, and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/6; 435/7.21

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

28 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

----- KWIC -----

Detailed Description Text - DETX:

In certain embodiments, the organism is an archaea (a.k.a. archaeobacteria; e.g., a methanogens, a halophiles, a sulfolobus). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a sulfolobus, a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiliaes, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

#### Detailed Description Text - DETX:

A preferred adjuvant in the present invention is BCG BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without trehalose dimycolate. Trehalose dimycolate may be used itself. Azuma et al., (1988) show that trehalose dimycolate administration correlates with augmented resistance to influenza virus infection in mice. Trehalose dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

#### Detailed Description Text - DETX:

The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with trehalose dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with trehalose dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and trehalose dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and trehalose dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

#### Detailed Description Text - DETX:

Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, .gamma.-interferon, GM-CSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.



US-PAT-NO: 6403355

DOCUMENT-IDENTIFIER: US 6403355 B1

TITLE: Amylases

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hagihara; Hiroshi	Tochigi	N/A	N/A	JP
Kitayama; Kaori	Tochigi	N/A	N/A	JP
Hayashi; Yasuhiro	Tochigi	N/A	N/A	JP
Igarashi; Kazuaki	Tochigi	N/A	N/A	JP
Endo; Keiji	Tochigi	N/A	N/A	JP
Ozaki; Katsuya	Tochigi	N/A	N/A	JP

APPL-NO: 09/ 465519

DATE FILED: December 16, 1999

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	10-362487	December 21, 1998
JP	10-362488	December 21, 1998

US-CL-CURRENT: 435/202; 435/183 ; 435/200 ; 435/201 ; 435/69.1

ABSTRACT:

Described are liquefying alkaline amylases each having residual activity not less than 70% when treated at pH 10 and 45.degree. C. for 30 minutes in the presence of 1 to 100 mM of EDTA or EGTA; and a detergent comprising the same. Compared with the conventional amylases for a detergent, they have high chelating-agent resisting performance.

10 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

----- KWIC -----

Brief Summary Text - BSTX:

Among the liquefying amylases known to date, a liquefying .alpha.-amylase (WO90/11352) derived from the strain belonging to Pyrococcus sp. and an

.alpha.-amylase (WO96/02633) which is derived from the strain belonging to Sulfolobus sp. and is effective in the liquefying step of a starch are free from the influence from a chelating agent. These enzymes however have the optimum acting pH in a range of 4 to 6 and 2.5 to 4.5, respectively and do not act in the alkaline range so that they are not suited as a component of a detergent.

#### Detailed Description Paragraph Table - DETL:

TABLE I Strain KSM-K36 Strain KSM-K38 (a) Results of The strains K36 and K38 are bacilli having a size of 1.0 to 1.2 .mu.m .times. microscopic observation 2.4 to 5.4 .mu.m and 1.0 to 1.2 .mu.m .times. 1.8 to 3.8 .mu.m, respectively. They form an oval endospore (1.0 to 1.2 .mu.m .times. 1.2 to 1.4 micron) at the center or near the end of the cell. Positive in the Gram's stain. Having no acid resistance. (b) Growth in various media Since the present strain is alkaliphilic, 0.5% sodium carbonate is added to the medium employed in the following tests. Nutrient agar plate Good growth is observed. The Good growth is observed. The culture colony has a circular shape. It colony has a circular shape. It has a flat surface, but a rough has a flat surface and a smooth periphery. The color of the periphery. The color of the colony is pale earthlike color. colony is yellowish brown. Nutrient agar slant Growth is observed. Growth is observed. culture Nutrient broth culture Growth is observed. Growth is observed. Nutrient-gelatin Good growth is observed. No Good growth is observed. No stab culture liquefaction of gelatin is liquefaction of gelatin is observed. observed. Litmus milk No change is observed. No change is observed. (c) Physiological properties Reduction of a Reduction of a nitrate is positive. Reduction of a nitrate is positive. nitrate and Denitrification reaction is Denitrification reaction is denitrification negative. negative. reaction MR test Owing to the alkaline medium, Owing to the alkaline medium, judgment is impossible. judgment is impossible. V-P test Negative. Negative. Formation of indole Negative. Negative. Formation of hydrogen Negative. Negative. nitride Hydrolysis of starch Positive. Positive. Assimilation of It grows on a Christensen's It grows on a Christensen's citric acid medium, but not on a Koser's medium, but not on a Koser's medium and Simmon's medium. medium and Simmon's medium. Assimilation of It assimilates a nitrate but not an It assimilates a nitrate but not an an inorganic ammonium salt. ammonium salt. nitrogen source Formation of a colorant Formation of a pale yellow Negative. colorant on King's B medium. Urease Negative. Negative. Oxidase Negative. Negative. Catalase Positive. Positive. Range for growth Temperature range for growth is Temperature range for growth is 15 to 40.degree. C. The optimum growth 15 to 40.degree. C. The optimum growth temperature ranges from 30 to temperature is 30.degree. C. 37.degree. C. The pH range for growth is 9.0 to The pH range for growth is 8.0 to 11.0. The optimum growth pH is 11.0. The optimum growth pH is similar to the above. pH 10.0 to 11.0. Behavior to oxygen Aerophilic. Aerophilic. O-F test No growth is observed. No growth is observed. Assimilation of Assimilated are D-galactose, D-xylose, L-arabinose, lactose, glycerin, saccharides meribiose, libose, D-glucose, D-mannose, maltose, sucrose, trehalose, D-mannitol, starch, raffinose and D-fructose. Growth on a Grown at a salt concentration of 12%, but no growth at a salt salt-containing concentration of 15%. medium

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	472	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2003/02/10 08:32
2	L2	4827	trehalose	USPAT; US-PGPUB	2003/02/10 08:33
3	L3	49	1 and 2	USPAT; US-PGPUB	2003/02/10 08:33
4	L4	128	non adj reducing adj saccharide\$1	USPAT; US-PGPUB	2003/02/10 08:39
5	L5	21	1 and 4	USPAT; US-PGPUB	2003/02/10 08:40

PGPUB-DOCUMENT-NUMBER: 20020164723

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164723 A1

TITLE: Method of producing saccharide preparations

PUBLICATION-DATE: November 7, 2002

US-CL-CURRENT: 435/96

APPL-NO: 09/ 908395

DATE FILED: July 18, 2001

RELATED-US-APPL-DATA:

child 09908395 A1 20010718 parent continuation-of 09632392 20000804 US GRANTED parent-patent 6303346 US child 09632392 20000804 US parent continuation-of 09499531 20000210 US GRANTED parent-patent 6136571 US child 09499531 20000210 US parent continuation-of 09198672 19981123 US GRANTED parent-patent 6129788 US child 09198672 19981123 US parent continuation-in-part-of 09107657 19980630 US ABANDONED child 09107657 19980630 US parent continuation-in-part-of 08979673 19971126 US ABANDONED

#### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/632,392, filed on Aug. 4, 2000, now allowed, which is a continuation of U.S. patent application Ser. No. 09/499,531, filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. patent application Ser. No. 09/198,672, filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. patent application Ser. No. 09/107,657, filed on Jun. 30, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/979,673, filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference. [0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. [0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

PGPUB-DOCUMENT-NUMBER: 20020102696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102696 A1

TITLE: RECOMBINANT THERMOSTABLE ENZYME WHICH FORMS NON-REDUCING  
SACCHARIDE  
FROM REDUCING AMYLACEOUS SACCHARIDE

PUBLICATION-DATE: August 1, 2002

US-CL-CURRENT: 435/200,435/183

APPL-NO: 09/ 419305

DATE FILED: October 15, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued  
prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09419305 A1 19991015 parent continuation-of 08505448 19950721 US PATENTED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	190183/1994	1994JP-190183/1994	July 21, 1994
JP	189706/1995	1995JP-189706/1995	July 4, 1995